IMPROVING THE ASSESSMENT OF THIRST AND FOOTPAD DERMATITIS IN BROILER CHICKEN WELFARE MONITORING SCHEMES

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CHAPTER 1:

GENERAL INTRODUCTION

Nowadays, producers of poultry meat are rearing broiler chickens using intensive and artificial production systems. This can result in serious welfare problems for the broiler chickens. In most countries around the world, farm animals are viewed as conscious beings, at least to some extent, with interests of their own (FAO, 2005). Ethical and moral considerations are recognized increasingly as important factors of food production processes. The public is at present very engaged in food safety and animal welfare (Frewer et al., 2005).

The broilers that are currently used in our intensive production systems are selected for high growth rate and carcass yields, in particular with regard to the breast meat. Within a period of 50 years, growth rate has tripled while breast meat percentage has doubled (Havenstein et al., 2003). This artificial selection process in conjunction with the intensive housing conditions are recognized as the main factors that compromise the welfare status of broilers.

In order to be able to compare animal welfare levels in broiler houses, during transport or at the slaughterhouse, reliable and valid assessment schemes are needed. An example of an assessment scheme is the Welfare Quality® protocol for broilers (NEN-document, Welfare Quality®, 2009). Within this system, the overall status of broiler welfare is assessed using a large set of indicators. All measurements can be integrated into an overall welfare category. Hence, it can be used for comparing welfare levels between different systems, poultry houses or abattoirs.

The objective of this dissertation is to improve existing animal welfare assessment schemes for broiler chickens. The focus is on the development of an animal based measure for assessing thirst and on the evaluation of an automatic footpad dermatitis assessment system at the slaughter line.

Before describing the main animal welfare topics of this dissertation (thirst and footpad dermatitis) in more detail, an overall picture of the broiler sector and of animal welfare is provided.

1. The intensive broiler production system in Belgium

1.1 The broiler production chain

An overview of the broiler production chain is given in Figure 1 (VEPEK, 2012).

The broiler production chain with its multiple processes is well organized. The chain starts with the breeding sector where pedigree stock (pure lines) is kept in farms with a high biosecurity level (Zoons, 2004). The selection of the broilers' basic stock is dominated by a very limited number of international companies (e.g. Ross, Cobb, Hubbard, etc). These companies provide the parent stock to specialized farms were only the breeders are housed. These broiler breeders produce the hatching eggs for the broiler chicken industry. These eggs are brought to a hatchery and delivered as day-old chicks to the poultry houses (VEPEK, 2012). The majority of the broilers produced are the fast growing strains housed indoors. There are slower growing broilers and broilers kept with an outdoor range, although it is a minority of the broiler population in the EU.

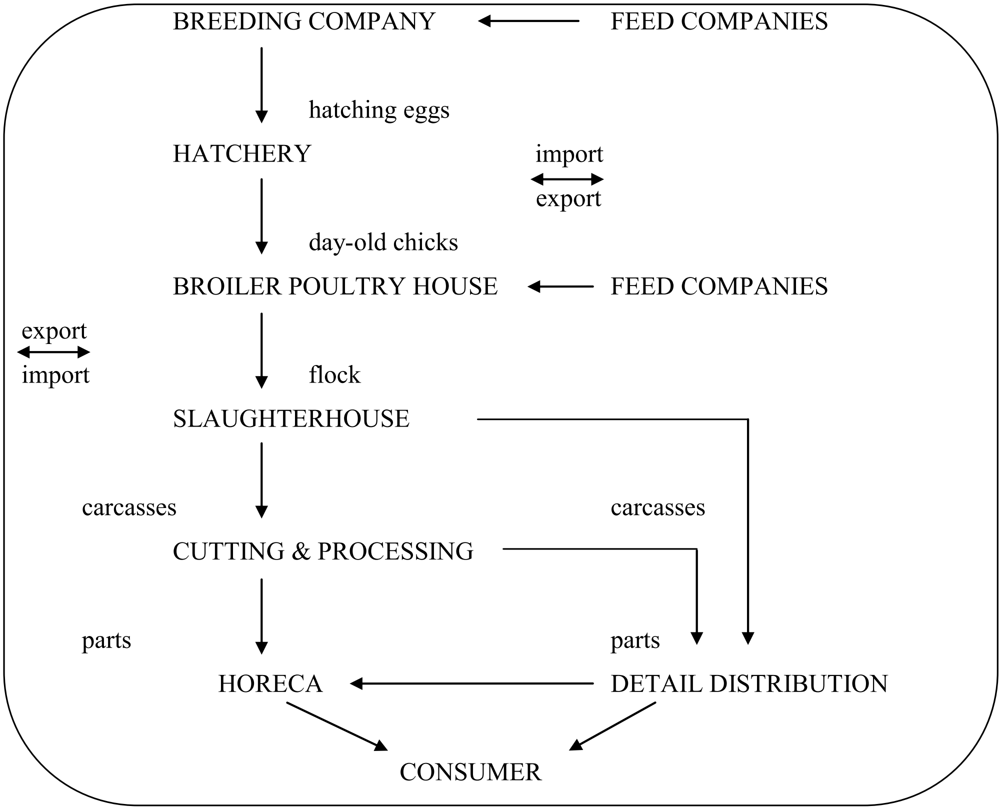

Figure 1 Overview of the broiler production chain (VEPEK, 2012).

The day-old chicks, intended for the broiler meat production, are transported to broiler farms. There they are usually housed in closed poultry houses where the environment is fully controlled. In these housing units broilers are kept in large groups, at high densities on littered floors where feeding and water provision is automated (Sørensen et al., 2006). After five to six weeks they are transported to a slaughterhouse from where the chicken meat (parts and/or whole carcasses) will be distributed. Feed companies supply the feed for the broiler breeder parent stock and for the broiler chickens.

The broiler industry in Belgium is a strongly integrated production system. It is mainly situated in Flanders, where 95% of all broiler chickens are produced on a contract basis. The integration occurs in Belgium predominantly through feed companies and hatcheries (and less through the distribution sector) (Vlaamse Overheid, 2006; FOD Economie, 2012).

1.2 Magnitude of the Belgian broiler production

In 2011 around 22 million broiler chickens places are present in Belgium with an average of 42,606 broilers per farm (FOD Economie, 2012; LARA, 2012). There are on average 6 to 6.5 broiler production cycles per year per production unit. Nearly 70% of these broilers are produced on farms with more than 30,000 birds (Table 1). An increase in scale can also be observed in the broiler slaughter industry. In 2010, 180 million broiler chickens were set up in Belgium. Ninety-two percent was slaughtered in only nine slaughterhouses. In 2011, an average price of 0.925 euro/kg was paid (VEPEK, 2012).

Table 1 Farm sizes of the Belgian broiler production in 2009 (source: VEPEK, 2012)

Production size: number of broilers per farm	**Broiler farm size category %**	**Broilers produced per farm size category %**
20 to 99	5.2	0.0
300 to 9,999	13.6	2.0
10,000 to 29,999	38.7	24.5
30,000 to 49,999	26.0	33.0
50,000 to 69,999	10.0	19.0
70,000 and more	6.5	21.5
TOTAL	100	100

1.3 Technical and economical aspects of the broiler production system in Belgium

The evolution (between 1998 and 2008) of several technical production parameters of the Belgian broiler industry is outlined in Table 2. Broilers now reach market weight in maximum six weeks, which is around one-third of the time it took 50 years ago (Havenstein et al., 2003). The average slaughter age of the birds in Belgium (although not continuously) has decreased from 41.3 to 40.1 days between 1998 and 2008. At the same time, the average slaughter weight has increased from 2.1 to 2.4 kg. Although slaughter weight increased, feed conversion ratio (i.e. amount of feed needed to obtain a kilogram of broiler meat) remained fairly constant at around 1.8. Mortality decreased with 1.9% to an average of 3.9% in 2008 (VEPEK, 2012).

In order to raise the birds with maximum efficiency, many conditions must be fulfilled, i.e. optimal temperature, humidity and ventilation, supply of good feed and water, stress prevention, and good sanitation (Van Horne et al., 2008; Vlaamse Overheid, 2012).

Table 2 Evolution of technical parameters on Belgian broiler farms within the period 1998 and 2008 (source: VEPEK, 2012)

Year	Slaughter age (days)	Average net weight (kg/bird)	Total mortality (%)	Average weight gain (g/bird/day)	Feed conversion (kg feed /kg bird)	Feed per animal (kg/bird)
1998	41.3	2.1	5.8	49	1.8	3.7
1999	42.0	2.1	4.6	50	1.8	3.9
2000	41.4	2.2	4.4	52	1.8	3.9
2001	41.0	2.2	4.3	54	1.8	4.0
2002	41.2	2.3	4.3	55	1.8	4.0
2003	41.2	2.3	3.7	56	1.7	4.0
2004	41.1	2.3	4.3	56	1.7	4.0
2005	41.1	2.4	3.6	56	1.8	4.1
2006	41.3	2.3	3.3	56	1.8	4.1
2007	41.6	2.4	4.9	56	1.8	4.2
2008	40.1	2.4	3.9	62	1.8	4.1

Feed cost, housing and labor are the main determinants of the competitiveness of livestock and meat-processing sectors (Van Horne et al., 2008). The income of a producer depends mainly on the price of day-old chicks, the feed costs, the average weight of the broilers and the price they receive from the slaughterhouse (Table 3) (LARA, 2010). Although the selling price of broiler meat steadily increased between 2005 and 2008, the income of the broiler producer showed a decrease in 2008, due to the high feed price. Nowadays, feed price is again high which compromises the profitability of the broiler producers because the selling price did not increase in the same way (FAO, 2012).

Table 3 Evolution of economical parameters of the Belgian broiler production between 2005 and 2008 (source: LARA, 2010)

	2005	**2006**	**2007**	**2008**
Income (euro/broiler)	0.29	0.34	0.40	0.22
Day-old chick (euro/chick)	0.26	0.26	0.27	0.31
Average weight broiler (kg/broiler)	2.31	2.38	2.42	2.41
Selling price (euro/kg broiler)	0.75	0.75	0.84	0.88
Feed price (euro/kg)	0.24	0.25	0.28	0.32

1.4 Legislative aspects of broiler housing

In June 2007 the Council Directive 2007/43/EG was published describing the minimum norms for the protection of chickens kept for meat production. This EU directive gives indications on management practices with particular focus on stocking density, light regimen, air quality, training and guidance for people dealing with chickens, as well as monitoring plans for holding and slaughterhouse.

Broiler poultry houses, holding more than 500 birds, should fulfill certain requirements concerning design and maintenance (CD 2007/43/EG):

- Drinkers shall be positioned and maintained in such a way that spillage is minimized;
- Feed shall be either continuously available or be meal-fed and must not be withdrawn from chickens more than 12 hours before the expected slaughter time;
- All chickens shall have permanent access to litter which is dry and friable on the surface;
- Ventilation shall be sufficient to avoid overheating and, where necessary, in combination with heating systems to remove excessive moisture;
- The sound level shall be minimized;
- All buildings shall have lighting with an intensity of at least 20 lux (at bird eye level) during the lighting periods, illuminating at least 80 % of the useable area. A 24-hour light rhythm with in total six hours dark, of which four hours are not interrupted, is required. Exception is made for the first seven days after arrival and three days before slaughter.

The broiler producer is further obliged to perform the following activities (CD 2007/43/EG):

- All chickens must be inspected at least twice a day, with special attention to signs indicating a reduced level of animal welfare and/or animal health. Those birds shall receive appropriate treatment or be culled immediately;
- After every production cycle, the buildings, equipment or utensils which are in contact with the chickens shall be thoroughly cleaned, and disinfected and clean litter must be provided;
- Records on introduced birds, their genotype, useable area, culling and natural mortality shall be kept for five years.

When stocking densities of more than 33 kg/m² are used in poultry houses, extra requirements need to be fulfilled. These requirements are detailed in the EU directive CD 2007/43/EG and, for Belgium specifically, in the Royal Decree of July 13, 2010.

This Council Directive states that if a stocking density of more than 39 kg/m² (with a maximum of 42 kg/m²) is used, the mortality shall not be higher than 1% + 0.06% times the age at slaughter in days (CD 2007/43/EG). Using the average age at slaughter in 2008 (see 1.3), mortality should not be higher than 3.4%.

2. International broiler production

Globally, the demand and supply of all types of poultry products have shown a rapid growth. Poultry production provides 28% of all meat consumed globally (Hancock, 2006 quoted by FAO, 2011). The production of poultry meat has grown over the past 40 years (1967-2007) with a factor of 7 (Table 4). The trade in poultry meat products has even increased with a factor of 30 (FAO, 2011).

In 2010, chicken meat was, globally, the fifth highest ranked commodity produced, after rice, cow milk, cattle meat and pig meat (FAOSTAT, 2010; FAO, 2011). The five biggest chicken meat producing countries are the United States of America, China, Brazil, Mexico and the Russian Federation. Belgium comes at number 39 in the ranking. Globally in 2007, poultry was the second most important meat product per person with a yearly consumption of 13.2 kg per head and is not far below that of pork meat (14.9 kg/head). Especially in Muslim countries, poultry meat is popular. The largest increase in global meat production is due to poultry meat followed by pig meat and to a much lesser extent beef meat

(Table 4). In Europe, chicken meat is ranked fourth in animal productions, after cow milk, pig meat and cattle meat (FAOSTAT, 2010). Belgian production contributes to the EU-production with 2.4% (VEPEK, 2012).

Table 4 Changes in the world livestock production from 1967 until 2007 (total and per head) (source: FAOSTAT)

Item	**Production (million tons)**			**Production per person (kg)**		
	1967	**2007**	**2007/1967**	**1967**	**2007**	**2007/1967**
Pig meat	33.86	99.53	294%	9.79	14.92	152%
Beef and buffalo meat	36.50	65.61	180%	10.55	9.84	93%
Eggs, primary	18.16	64.03	353%	5.25	9.60	183%
Milk, total	381.81	680.66	178%	110.34	102.04	92%
Poultry meat	12.39	88.02	711%	3.58	13.20	369%
Sheep and goat meat	6.49	13.11	202%	1.88	1.97	105%

Much of the global trade in chicken meat is explained by variations in consumer preferences across the world. Producers export the cuts to the markets where they get the best price, e.g. the EU largely prefers breast cuts and the legs and wings are exported to the Russian Federation (Dyck et al., 2003).

The most complete projections indicate a significant growth in poultry meat consumption from 2010 to 2051. The FAO expects the highest growth in poultry compared to other meat types: 2.3 times more poultry meat (as compared to 1.4 and 1.8 times as much for other livestock products) will be consumed in 2050 versus 2010 (Table 5) (FAO, 2011; FAO, 2006). Thus, it is expected that between 2020 and 2030 poultry meat will have become the first animal meat production in the world.

Table 5 Projected total consumption of meat and dairy products (source: FAO, 2011)

	2010	2020	2030	2050	2050/2010
			(million tons)		
WORLD					
All meat	268.7	319.3	380.8	463.8	173%
Bovine meat	67.3	77.3	88.9	106.3	158%
Ovine meat	13.2	15.7	18.5	23.5	178%
Pig meat	102.3	115.3	129.9	140.7	137%
Poultry meat	85.9	111.0	143.5	193.3	225%
Dairy not butter	657.3	755.4	868.1	1038.4	158%
DEVELOPING COUNTRIES					
All meat	158.3	200.8	256.1	330.4	209%
Bovine meat	35.1	43.6	54.2	70.2	200%
Ovine meat	10.1	12.5	15.6	20.6	204%
Pig meat	62.8	74.3	88.0	99.2	158%
Poultry meat	50.4	70.4	98.3	140.4	279%
Dairy not butter	296.2	379.2	485.3	640.9	216%

2.1 Advantages and disadvantages of the broiler production system compared to other meat production systems

Chickens are monogastric animals that require nutrients quite similar to that of a modern human diet. Their diet in industrial production units includes cereals and protein sources (mainly soya), fishmeal, but also roughage. They have been bred to become the most efficient feed converters (FAO, 2011) and therefore, poultry meat, compared to other meat products, is a very efficient way to convert grains and cereal by-products into animal proteins (AVEC, 2011).

While chicken prices remain competitive and preferred by price-sensitive consumers, difficulties in passing off higher feed costs have resulted in negative profit margins for the sector (FAO, 2012). Intensive systems have the advantage of economies of scale that make it possible to produce livestock protein in large quantities in a relatively cheap way. For a growing urban population this is an important consideration. The less-intensive and extensive systems are an excellent option for rural populations with access to short food chains and for consumers who can afford to buy "green" products (FAO, 2011).

FAO confirmed that poultry meat has, among all meat types, the most favorable image with regards to the carbon footprint (Gerber et al., 2008). Due to an efficient digestion process and the absence of enteric fermentation, poultry meat is placed as the most sustainable meat in terms of low values of greenhouse gas emissions. In terms of carbon footprint and land use, per kilo of product, life cycle assessment studies show that the production of poultry compared to other meat producing species emits less CO_2 and uses less land area (m^2/kg) compared to other animals (AVEC, 2011).

A strong increase in average growth occurred in the broiler sector due to genetic selection for improved performance. Unfortunately, intense and disproportionate selection for individual production-related traits starts to have an increasingly negative impact on the animals' well-being. In the case of genetic selection for fast growth in chickens, published research clearly demonstrates that this point has been reached and even passed (EFSA, 2010).

3. The welfare of broiler chickens

3.1 Definition of animal welfare

Animal welfare has been defined in literature in many different ways. The long debate on animal welfare includes the difficulty of defining the term animal welfare itself (Carenzi et al., 2009). It is important that the definition reflects a clear concept, which can be scientifically assessed (EFSA, 2006). Not only science, but also politics, religion and culture influence the definition of animal welfare (Swanson, 1995). Some confusion exists also between the terms welfare and well-being, which in dictionaries are respectively: "the state of being or doing well" and "a good or satisfactory condition of existence," which are linked by the concept of 'quality of life' (Fraser, 1998). In this dissertation the term 'welfare' is used. Fraser (1998) developed several views on how people look at animal welfare. While some people emphasize how animals feel, others emphasize the biological functioning of the animal. In the past, veterinarians and farmers have seen animal welfare predominantly in terms of the body and the physical environment. A third view on welfare, linked to the feeling-based approach, is that animals fare best if they can live according to their natural condition. This approach emphasizes that animals should be kept in reasonably natural environments (Hewson, 2003; Fraser, 1998).

Many attempts have been made to propose a more scientific definition. Currently, the most commonly used definition is the one based on the "Five Freedoms for Animal Welfare" by UFAW in 1993 (Based on Brambell, 1965). These freedoms define the following needs of animals that should be met under all circumstances:

- Freedom from hunger and thirst;
- Freedom from pain, injury and disease;
- Freedom from thermal and physical discomfort;
- Freedom from fear and distress;
- Freedom to express normal behavior.

According to Broom (1986) animal welfare, which varies from very poor to very good, can be scientifically assessed by combining indicators of the animal's physical and mental status. Good welfare can occur provided the individual is able to adapt to, or cope with, the constraints it is exposed to (Broom, 1986). For some people, this is rather a definition that refers to measurable parameters of biological functioning, e.g. survival, normal behavior, physiology and reproductive success. The five freedoms define animal welfare more in terms of subjective emotional states, and this definition is more used these days (European Parliament, 2009).

Vanhonacker et al. (2012) conducted a study on conceptualizing farm animal welfare, incorporating the public's perception and integrating the opinion of different stakeholder representatives. The resulting conception revealed seven dimensions grouped in two different levels (Figure 2).

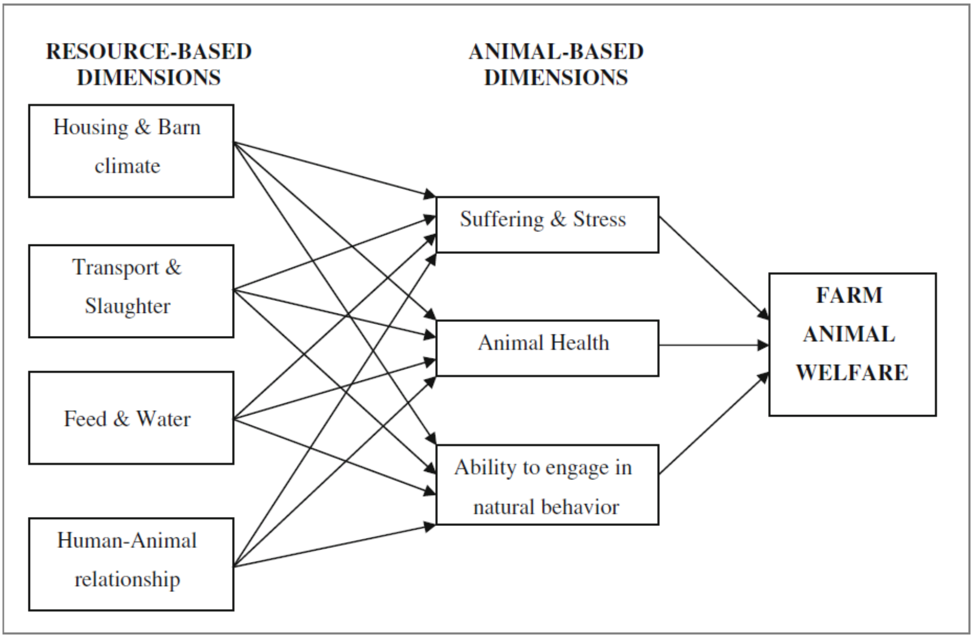

Figure 2 The farm animal welfare conception according to Vanhonacker et al. (2012)

Animal welfare is a complex and multidimensional concept and the operating definition should state clearly which dimensions are relevant, so that a complementary set of indicators can be selected and aggregated in order to assess the overall animal welfare status (Tuyttens et al., 2010). In this dissertation we focus on the operational definition of animal welfare proposed by Welfare Quality® (explained in section 3.6).

3.2 Animal welfare and the broiler production system

From 1950 onwards, animal production systems intensified due to economies of scale, specialisation and mechanisation (De Tavernier, 2000; FAO, 2005). The broiler production sector evolved from millions of small backyard flocks, where meat was a by-product of egg production, to a very big industry (HSUS, 2008). Animal welfare in commercial poultry production systems became an important topic in Europe. But this awareness is also rising in other parts of the world. In some countries, e.g. Brazil, this interest is mainly driven by export opportunities for poultry meat, especially to Europe (HSUS, 2008).

Public concern on broiler welfare and the bad image of this sector are not entirely in contradiction with scientific findings that considerable welfare problems do exist in the different stages of the production process. This dissertation will discuss welfare problems during the rearing period and at the end of the rearing period and before slaughter.

Over the years, broiler chickens have been selected for their rapid growth rate as well as for high carcass yields, with particular attention to the breast (EFSA, 2012; SCAHAW, 2000). Meluzzi et al. (2009) reported that the intensive genetic selection made the present broiler chickens the fastest growing farmed species. The major animal welfare problems identified in the SCAHAW report (2000) are mainly based on the intense and almost exclusive selection in breeding on growth and feed conversion. The Humane Society of the US reports that between one-third and one-half of the broilers suffers from leg deformities and one-quarter suffers from chronic pain (HSUS, 2008).

In the intensive production units, broiler chickens are reared at stocking densities ranging from 22.5 to 42.5 kg live weight/m² (SCAHAW, 2000). 42.5 kg/m² is not allowed anymore in the EU since the new legislation on the protection of chickens kept for meat production as stocking density was thought to be the major factor for poor welfare status (Meluzzi et al., 2009). However, Dawkins et al. (2004) claimed that housing conditions, e.g. litter quality, temperature and the humidity within the poultry house, are more important than stocking density itself.

The main welfare problems in broiler chickens described in SCAHAW (2000) and the EFSA report (2012) include high mortality rates, skeletal and muscular disorders, contact dermatitis, ascites and Sudden Death Syndrome, respiratory and mucous membrane problems, stress, thermal discomfort and behavioral restrictions.

The end of the broiler production cycle is a particularly stressful period in the broilers' life (May et al., 1989). At this point, the density (kg/m²) reaches its maximum followed by the stressful catching and transport to the slaughterhouse. The last day in the poultry house, a withdrawal period for feed and water takes place as to minimize carcass contamination (May et al., 1989). Feed withdrawal times of 4 to 5 hours prior to catching have been recommended (Bilgili, 2002). During this period, water is still provided as to facilitate the clearance of feed. Water is usually withdrawn just before the first bird of a flock is caught and crated for transport to the slaughterhouse (Bilgili, 2002; Warriss et al., 2004).

Broiler chickens are transported from the poultry house to the slaughterhouse. Transporting broilers involves placing them into transport containers that are loaded on to the lorries and brought to slaughterhouses. The process of catching, loading, transporting and unloading must be done at a rate appropriate to the line speed of the slaughterhouse. Otherwise efficiency will suffer and costs will rise (Delezie, 2006). The depopulation process often takes several hours with a crating rate of 1,000 to 1,300 broilers per hour per catcher (Delezie, 2006).

During transport, varying degrees of stress are brought upon the birds (e.g. thermal stress, crowding, motion, social disruption and noise) (Mitchell et al., 2008; Mitchell, 2009). The whole process of catching, loading, and transport causes many problems such as serious injuries, metabolic exhaustion, dehydration, thermal stress and may even lead to death (CIWF, 2005; Delezie, 2006; EFSA, 2011). An average of 0.126% is presented by Warris et al. (2005) as mortality in transport or dead on arrival.

3.3 Sensitivity of consumers to animal welfare

European legislation dealing with animal welfare reflects strong public concern of European citizens about animal welfare (Moynagh, 2000). Consumers have increasing preference for better animal welfare products (Moynagh, 2000; European Commission, 2006). Unfortunately, empirical evidence suggests that consumers' willingness to pay for products subject to high animal welfare standards is relatively low in the market (Theuvsen et al., 2005).

Consumers' statements do not always translate into actual purchasing behaviors. According to Vanhonacker et al. (2009) the difference lies in the fact that individuals tend to respond to questionnaires as citizens. But when a choice has to be made as a consumer, the individual is not equally willing to pay. Consumers and citizens have different interests. A consumer prefers healthy, tasty and cheap food that is easily available. A citizen prefers the same characteristics as described for a consumer and also that the production methods are ethically acceptable. Animal welfare does not receive the same importance in the consumption pattern.

Moynagh (2000) believes that EU consumers are willing to pay considerably more for welfare-friendly production practices, but not when the broiler meat is packaged as convenience food (i.e. prepared meals). Convenience foods are too far removed from the live animal to be directly linked to welfare issues. This is the "commodity politics" of animal welfare. From the study of Vanhonacker et al. (2009) a welfare label emerged as an appropriate communication vehicle for consumers who are engaged pro-welfare. Consumers appreciated the label for higher welfare products.

3.4 Producers' view on animal welfare

Animal welfare is increasingly referred to as an important goal by different stakeholders along the food production chain (Verbeke, 2009), yet its meaning and conception differs depending on who is using the concept (Vanhonacker et al., 2012). Producers' (as well as retailers') view on animal welfare is primarily commercially, economically or politically orientated (Verbeke, 2009). Retailers aim more at attracting particular consumer groups (Anwander Phan-Huy et al., 2003). Livestock producers tend to focus predominantly on performance and reproduction parameters and strive to maintain or expand their market share (Anwander Phan-Huy et al., 2003; Bracke et al., 2005, Vanhonacker et al., 2008). Vanhonacker et al. (2008) compared Flemish farmers' interpretations of the concept of farm animal welfare with those held by Flemish citizens. Farmers and citizens had to give the importance of 72 aspects relating to animal welfare on a scale. The producers did attribute a lower importance to most of these aspects than the citizens, and they had a more positive view of the current situation with regard to farm animal welfare, in particular for those aspects relating to natural behavior, pain, stress and availability of space. The largest perceptual discordance was found for aspects relating to the ability to engage in natural behavior. Citizens were much more concerned about these aspects than farmers. European farmers worry about the costs of welfare assessments and improvements, and also about more stringent regulations because of the globalization of trade and production (Bock, 2009). Farmers seem to favor a common approach towards animal welfare throughout Europe and preferably worldwide (Blokhuis, 2009).

3.5 Animal welfare and trade legislations

Animal welfare in broiler production systems receives more legislative attention in the EU than in many other regions worldwide (Matheny et al., 2007; Van Horne et al., 2008). Such legislation is mostly science-based and anteceded by scientific reviews by for example SCAHAW, the EU Scientific Committee regarding Animal Health and Welfare (Moynagh, 2000). Unfortunately, these legislative norms often cause a cost-handicap as they increase production costs (Theuvsen et al. 2005). The production costs for broiler meat when applying existing European broiler welfare rules in 2006, increased with 10% (Grethe, 2007).

At present, the World Trade Organization prevents and eliminates trade barriers like e.g. import bans. As animal products produced outside the EU and with less stringent animal welfare legislation are physically identical to the EU-products, their import into the EU market cannot be banned. Van der Straeten (2011) argued that if the EU would be allowed to impose such rules on production methods for imported broiler meat, this could stimulate importing countries to enhance animal welfare. The increased production costs for adhering to the higher standards will often be higher for developed than developing or growing countries, where the costs for labor and housing tend to be lower. An upgrade of EU regulation requirements for animal welfare in imported broiler meat would operate rather as an opportunity to create additional added value. Brazil, for example, can currently compete in the European market due to very low production costs (Van Horne, 2008).

To summarize, although agricultural production focused mainly on supply, price and competition, these days European consumers expect their food to be produced and processed with greater respect for the welfare of the animals (Blokhuis et al., 2003). The study from Vanhonacker et al. (2012) acknowledges the importance that the chain end-user can play in the debate on animal welfare. Animal welfare, however, is still considered a non-trade concern at WTO-level. Campaigns directed at consumers and retailers are likely to be more cost-effective than production-related regulations in improving animal welfare (Matheny et al., 2007).

In order to accommodate societal concerns about animal welfare of food products as well as related market demands, there is a pressing need for reliable systems to assess the animals' welfare status (Blokhuis et al., 2003; Kjaernes et al., 2007). Tools for assessing animal welfare are needed in order to be able to compare different welfare levels in the poultry houses, at the slaughterhouses or during transport. These tools will be discussed in paragraph 3.6.

3.6 Tools to assess animal welfare in broiler production systems

It is common knowledge among broiler chicken producers around the world that in order to reach maximum efficiency, good conditions must be provided allowing the broilers to express their maximal production potential and by consequence a basic level of animal welfare is ensured. However, it has already been longtime recognized that good productivity and health are not necessarily indicators of good welfare (Jones, 1996). Housing parameters related to structures, design and micro-environment, even if they are reliable and easier to measure, can only identify conditions which could be detrimental to animal welfare. Housing parameters cannot predict poor welfare in animals per se (Sevi, 2009; Blokhuis, 2012).

Animal welfare science deals with the animal's welfare status from the animal's perspective which, unfortunately, cannot simply be asked to the animals. Many attempts have been made to characterize and assess animal welfare starting from the animal's point of view, and to provide data for ethical decisions about appropriate animal environments (Dörfler, 2007).

Increasingly the whole organism is considered in the totality of its environment (Magiels et al., 2004). For an adequate assessment of welfare a wide range of indicators must be used, although single indicators can show that welfare is poor (SCAHAW, 2000).

In the Welfare Quality project a new approach with preference given to animal-based indicators (Gloor et al., 1985; Leeb et al., 2001; Whay et al., 2003) was used. The protocols developed by Welfare Quality® are characterized by a hierarchical integration method by which the data on the various welfare measures can be aggregated into an overall welfare category. Those specific measures can also be used by farmers to improve the welfare of their livestock. This welfare qualification system can be used to inform consumers about the welfare quality of food products. Using this system, standardized on-farm animal-based welfare assessment is becoming technically feasible (European Parliament, 2009). The feedback to farmers regarding the outcomes of welfare assessments is necessary for on farm welfare management. Such information, together with expert advice on causal problems and possible improvement of strategies, can support the farmers' efforts to further improve the animal welfare status (Blokhuis, 2012).

Welfare Quality® has also developed a special protocol for broiler assessment.

The focus of this dissertation is on the animal welfare definition developed by Welfare Quality® and the associated standardized assessment method for broiler chicken welfare. Welfare Quality® defines animal welfare in terms of four main principles: (1) good feeding, (2) good housing, (3) good health and (4) appropriate behavior (Table 6). In turn, these four principles encompass 12 criteria, each covering a separate aspect of welfare. Botreau et al. (2007) explained that the set of criteria should be exhaustive (no missing items), minimal (only necessary items), agreed by stakeholders, and legible (a limited number of criteria). Furthermore, the interpretation from one criterion should not depend on that from another (Botreau et al., 2007). They were defined through combined analyses of consumer perceptions and attitudes with existing knowledge from animal welfare science.

The welfare assessment protocol for broilers (Welfare Quality®, 2009) describes measures indicative of broiler welfare on-farm, as well as measures indicative of broiler welfare during transport and slaughter. The different indicators needed for assessing broiler welfare according to the Welfare Quality system are given in Table 6. Within the Welfare Quality® assessment system, some resource- and management-based measures are still used, but only if an appropriate (in terms of sensitivity, validity, reliability and feasibility) animal-based measure was not available.

For most criteria, there is at least one measure defined to evaluate the corresponding criteria. Some measures can only be assessed on-farm or at the slaughterhouse, but for others one can choose. The criterion *Absence of pain induced by management procedures* does not apply for broiler chickens, since no procedures inflicting pain on broilers are systematically included in the broiler production management (Welfare Quality®, 2009). For the criterion *Expression of social behaviors*, unfortunately, no measure, as yet, is developed (Welfare Quality®, 2009).

Table 6 Broiler welfare assessment protocol (Welfare Quality, 2009).

Principle	**Criteria**		**Measures**
Good feeding	1.	Absence of prolonged hunger	Emaciation
	2.	Absence of prolonged thirst	Drinker space
Good housing	3.	Comfort around resting	Plumage cleanliness, litter quality, dust sheet test
	4.	Thermal comfort	Panting, huddling
	5.	Ease of movement	Stocking density
Good health	6.	Absence of injuries	Lameness, hock burns, footpad dermatitis, breast burns
	7.	Absence of disease	On farm mortality, culls on farm, ascites, dehydration, septicaemia, hepatitis, pericarditis, abscess
	8.	Absence of pain induced by management procedures	*Not applied in this situation*
Appropriate behaviour	9.	Expression of social behaviours	*As yet, no measure developed*
	10.	Expression of other behaviours	Cover on the range, free range
	11.	Good human-animal relationship	Touch test
	12.	Positive emotional state	Qualitative Behaviour Analysis (QBA)

3.7 Attention points in using animal welfare assessment tools for broiler chickens

A golden standard for the true overall welfare status of an animal does not exist. Welfare Quality® developed an integration tool to aggregate the various welfare measures into an overall welfare category. In such an aggregated measure, certain decisions have to be taken. For instance, it needs to be decided whether the average state of the animal or the worst observed state is considered, whether each welfare criterion is considered separately or together in a more holistic approach (Veissier et al., 2011). Welfare Quality® consulted experts (i.e. animal scientists, social scientists and stakeholders) to support such decision making and the methodology for an overall assessment was adjusted according to their opinions. The chosen measures should be valid (an actual reflection of the animal's welfare), reliable (repeatable within and between different persons and on different occasions) and feasible (must be possible to implement them in practice and at a reasonable cost) (Keeling, 2008). Initial and ongoing training of assessors in the field and the abattoir is very important to ensure the reliability of these measures (EFSA, 2012).

The proposed duration of the evaluation in the protocol has received most criticism. The long duration is the bottleneck for a large-scale application and makes an assessment costly. On average, the broiler welfare assessment protocol on-farm takes about 3 to 4 hours per flock (Welfare Quality®, 2009). Stakeholders already expressed their concern on the amount of time needed to perform the assessment protocols (Manten et al., 2001). The need for the development of less labor intensive systems is high. One possibility is to investigate whether the assessment could be reduced by 1) reducing the number of animals that need to be assessed individually or the duration of group assessments, or by 2) omitting certain measures (preferably those which take time) (e.g. gait is highly correlated with footpad dermatitis and therefore only one of them needs to be assessed). Another possibility is to improve efficiency by no longer performing the assessments on farm, but at the slaughterhouse. The Welfare Quality® assessment protocol provides the possibility to collect data at the slaughterhouse, which may replace the necessity for collection of certain data (e.g. footpad dermatitis and hock burn) on farm (De Jong et al., 2011a). If slaughterhouse and on farm measures are closely related, replacement of on farm assessments with slaughterhouse measures will reduce assessment time considerably (De Jong et al., 2011a).

Although the Welfare Quality® protocol for broiler chickens contains reliable, valid and feasible indicators, some aspects can still be improved. The thirst criterion has been criticized. Furthermore, automatic assessment of some criteria, such as footpad dermatitis, can increase the number of animals assessed and eliminate the subjectivity of an assessor. This dissertation is focused on the development and validation of an animal-based indicator for thirst in broilers and on the evaluation of a system that automatically scores footpad dermatitis of broilers at the slaughter line.

3.8 Thirst

Thirst is a subjective perception that motivates animals to drink (Sprenger et al., 2009). Jones et al. (2009) describe that prolonged thirst causes stress and, if long-lasting or severe, leads to debilitation, loss of body condition and disease (Jones et al., 2009). It also reduces food intake, which in turn may cause hunger.

Why investigating thirst?

One of the five freedoms defined by the UFAW (1993), *freedom of thirst* is considered to be of paramount importance for animal welfare (Brambell, 1965; Vanhonacker et al., 2008; Tuyttens et al., 2010). Within the Welfare Quality® integration, a relatively high weight has been assigned to thirst, because it belongs to a principle with only one other criterion (Tuyttens et al., 2010). Vanhonacker et al. (2008) indicated that Belgian farmers and citizens thought that thirst was not a problem within the production system. However, whether or not thirst is a welfare problem in broiler production systems, can only be determined by a proper animal-based indicator. In existing broiler welfare evaluation schemes, freedom from thirst is assessed by a resource-based indicator such as the number of animals per drinker, checking possible leaks in the drinker line and by checking drinker alarms (Sprenger et al., 2009). Although such resource-based measures can often be assessed quickly and have good inter- and intra-observer reliability, they may not be very sensitive and accurate measures of thirst. As discussed earlier (paragraph 3.6) these resource-based measures should be interpreted as risk factors for a certain welfare outcome, in this case thirst. This welfare outcome ideally should be assessed using an animal-based measure. However, Welfare Quality® resorted to resource-based measures of thirst because they failed to identify or develop an animal-based measure of thirst (Algers, 2006) that met the criteria with regards to validity, reliability and feasibility. The EFSA report (2012) mentions several thirst indicators without information on validity or reliability of these measures.

Problems with thirst

Birds have free access to water (automatically distributed) throughout their life in a poultry house (Meluzzi et al., 2009). Jones et al. (2009) listed three main causes of prolonged thirst:

- Poor water quality or insufficient/inadequate drinking facilities (mainly due to neglect or poor husbandry). Water availability may be inadequate in extensive broiler production conditions;
- Competition with conspecifics when water is limited (e.g. insufficient drinker space);
- Long distance transport (water is withdrawn before the birds are transported (Bilgili, 2002)).

Broiler producers need to be well trained to avoid thirst problems due to poor husbandry and neglect (Jones et al., 2009). The water feed ratio (provided by breeder companies) is used by broiler producers as a monitoring tool for evaluating the flock's water consumption in the poultry house (Watkins et al., 2009). The general belief is that thirst is unlikely to be a common problem in the intensive broiler production system since dehydration reduces production performance. This may explain the paucity of research on this subject and the lack of validated measures of thirst (Sprenger et al., 2009).

No individual dehydrated broilers are detected on-farm by looking at resource-based measures like the water feed ratio (birds consume approximately 1.6 to 2 times as much water as feed (Watkins et al., 2009)). Individuals within large groups that are severely thirsty (whatever the cause might be) may go unnoticed. CIWF reported (2005) the possibility of some broilers being unable to reach drinkers due to lameness. Locomotion problems, diseases and high stocking densities likely increase the risk of thirst (SCAHAW, 2000). Animals with locomotion problems reach the drinkers less easily and therefore might suffer thirst. High stocking density and inappropriate designs of poultry houses can make it more difficult for animals to access water. A study from Feddes et al. (2002) showed that water consumption per chicken decreases with increasing stocking densities and only tended to decrease with increasing water nipple scarcity. As for disease, small or very weak animals are at risk as for their inability to reach the drinkers, and are likely to die. The reduced water consumption appeared to be independent of the effect of stocking density on feed consumption (Feddes et al., 2002).

Drinker related issues

Drinker types

Commercially reared broiler chickens are frequently supplied with water through lines of nipple drinkers (Picture 1), which the birds have to peck or press to release water (Houldcroft et al., 2008). To avoid water spillage and the consequent spoiling of the litter underneath, the nipple line is positioned above the birds' heads and gradually raised as the birds grow. Sometimes a cup is fixed underneath the nipple drinker for minimizing litter spoilage. Bell drinkers (Picture 2) are sometimes used as an alternative to nipple drinkers and allow birds to drink more naturally (Appleby et al., 2004). A third system that is sometimes used is a cup drinker (Picture 3). This system combines the previous two systems. Birds have to peck or press a nipple enclosed in a cup to release water.

Picture 1 Nipple drinker

Picture 3 Cup drinker

Picture 2 Bell drinker

When a nipple drinker is used, water drips down into the birds' throats. This, however, means that the action of taking in water is different from the natural behavior of drinking. According to Houldcroft et al. (2008) the natural drinking behavior of a bird includes the 'scoop action', in which the bird lowers its head, takes water into its bill and then raises its head again. This 'scoop action' is completely missing in the frequently used nipple drinker system.

Apart from the inability to reach or operate the drinker, other factors might also cause thirst (SCAHAW, 2000). Given the choice, birds prefer open drinkers to high drinking nipples (Houldcroft et al, 2008). A disadvantage of open drinkers compared to drinking nipples is the higher degree of water spillage resulting in poor litter quality (De Jong et al., 2012). Wet litter could result into the following welfare consequences for broilers: irritation of the respiratory tract and eyes due to atmospheric ammonia and pain induced by hock burns, footpad dermatitis and breast burns (EFSA, 2012). For this reason, most commercial broiler production systems use nipple drinkers (May et al., 1997). Nipple lines are heightened with the age of the chickens and, therefore, the chickens need to stretch their neck to reach the nipple, which is an unnatural drinking posture for a bird (Houldcroft et al, 2008). In a study by Houldcroft et al. (2008) many birds started drinking "as if they were somewhat dehydrated" when nipple height was lowered. The nipple cup system could be a better alternative, since drinking posture is more natural and it allows easy access to water and also minimizes spillages (De Jong et al., 2012).

3.9 Footpad dermatitis

What type of lesion is footpad dermatitis?

Footpad dermatitis is a type of contact dermatitis where lesions appear on the plantar regions of the broilers' feet (Greene et al., 1985). Dermatitis can also appear on the hocks (hock burn) and the breast (breast burn) of birds (Martland, 1985). The lesion on the footpad probably causes the birds to sit on their breasts for long periods, resulting in the development of breast and hock lesions (Harms et al., 1975; Martland, 1985; Allain et al., 2009). The lesions on breast and hocks usually develop more slowly and are less frequent than lesions on the feet (Stephenson et al., 1960). All lesions may occur together in a single bird (Haslam et al., 2007). They may be mild, showing only hyperkeratosis and discoloration (Ekstrand et al., 1998). In severe cases, swelling and erosions or ulcers can be seen (Martland, 1985). This type of lesion can heal, but rarely does under commercial conditions because of a combination of moisture and chemical irritants in the litter (Greene et al., 1985; Martland, 1985; Berg, 2004). If footpad lesions arise at a young age they may heal if litter quality significantly improves (Martland, 1985), which rarely happens under commercial circumstances. It only happens when flocks are thinned, as a portion of the flock is removed to the slaughterhouse (SCAHAW, 2000). Healed lesions can be observed in commercial flocks.

Causes of footpad dermatitis

Among the many factors that influence the incidence of footpad dermatitis, SCAHAW (2000) included feed compositions (e.g. methionine deficiencies) and seasonal effects (e.g. varying relative humidity influencing litter quality). Haslam et al. (2007) reported a higher incidence in winter, and found an association between footpad dermatitis prevalence and the feed supplier. Veldkamp et al. (2007) reviewed the literature and listed the following possible influencing factors: gender, chick origin, age, health, water supply, feed composition, litter type, litter depth, top dressing, climate and light. However, caution is needed because for some of these factors, the effect on footpad dermatitis could not be reproduced by other scientists (De Jong et al., 2012; Meluzzi et al., 2009; Veldkamp et al., 2007; SCAHAW, 2000). It seems that management practices are the most important factors for preventing the occurrence of wet litter, since wet litter, together with feed consumption, is believed to be the main underlying factor of the disease (SCAHAW, 2000). This was recently confirmed in a large scale study in which dietary protein content and ventilation scheme were studied (Maertens et al. 2012; Löffel et al., 2012). A too low ventilation rate in wintertime induced a significantly higher prevalence of broilers with severe footpad and hock lesions. Also the relationship between the dietary protein content and the prevalence of footpad dermatitis was clearly demonstrated (Maertens et al., 2012).

Footpad dermatitis is an important aspect of animal health and welfare in the broiler production system and is a relatively widespread problem in the European broiler production system (SCAHAW, 2000). In severe cases, footpad dermatitis causes pain to the bird which leads, together with a decreased health, to a welfare problem (Veldkamp et al., 2007; Berg, 2004). Apart from the animal welfare aspects, footpad dermatitis is also a concern for the production performance. Footpad dermatitis has an impact on the technical results (e.g. slower weight gain) and the slaughter quality (e.g. higher rejection percentages) (Cengiz et al., 2011; Dowsland, 2008; Veldkamp et al., 2007; SCAHAW, 2000).

Footpad dermatitis as indicator for broiler welfare

Footpad dermatitis was originally included as a welfare parameter in the draft versions of the European animal welfare Directive (CD 2007/43/EG) but was not retained in the last version (Meluzzi et al., 2009). The idea was to include footpad dermatitis as an indicator for the animal welfare level of the flock. If within a flock the prevalence of footpad dermatitis is high and this problem continues, a broiler producer would be *punished* by a mandatory decrease of the maximum stocking density in the following flock (Veldkamp et al., 2007). Footpad dermatitis assessment may well be not included in the European animal welfare Directive but it is, already since the nineties, routinely used within broiler welfare monitoring programs in Sweden and Denmark. In these countries, veterinary inspectors routinely measure the incidence and severity of this lesion in all flocks (De Jong et al., 2011b; Veldkamp et al., 2007). This information is gathered at the slaughter line and is used to increase the awareness of broiler producers about this welfare problem and the situation in their flocks. Moreover, it also determines the maximum stocking density permitted on their farm (Ekstrand et al., 1997). In July 2012, the Netherlands also started linking the stocking density to a norm for footpad dermatitis. The government's aim was to reduce footpad dermatitis prevalence and to address this problem on Dutch farms (De Jong et al., 2011b).

Automated measuring

In 2008, WUR Livestock Research, Meyn Food processing Technology and Flandrex joined forces to develop a camera system for assessing footpad dermatitis automatically at the slaughter line. The assessment is based on video imaging of the footpads. An existing imaging technique currently used at different slaughterhouses for carcass classification and for identification of carcass damage, was further developed to be used for assessing footpad lesions (De Jong et al., 2010). This automatic video imaging has several advantages compared to a manual assessment. It is more objective, more efficient, is able to assess bigger sample sizes compared to a manual assessment of a flock and an automatic feedback system is possible (De Jong et al., 2011b). New software has been developed using the Swedish scoring system for footpad lesions (class 0, no lesions; class 1, moderate lesions; class 2, severe lesions) (De Jong et al., 2010). The first prototype was already assessed by De Jong et al. (2011) with the support from Welfare Quality®. Images could be made of 95.8% of the feet that passed the camera system (De Jong et al., 2008). The feet were hung on the slaughter line of a commercial slaughterhouse, using the normal line speed. However, the software failed in scoring feet in class 1 (moderate lesions), because only 16.7% agreement with the expert scores was obtained for the feet in class 1 (De Jong et al., 2008). They concluded that the automatic system looks promising, but some further improvements on the software were needed before the automatic system could be used in practice.

This dissertation focuses on the development of an animal-based indicator for thirst and on the evaluation of this automatic footpad dermatitis system.

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CHAPTER 2:

RESEARCH OBJECTIVES

The general objective of this dissertation is to improve existing animal welfare assessment schemes for broiler chickens, such as the Welfare Quality® scheme.

Two important aspects of the Welfare Quality® scheme relate to thirst and footpad dermatitis. Both aspects are studied in detail in this dissertation, in order to propose better alternatives for the assessments that are currently done.

The first objective related to thirst is to identify a reliable and fast animal-based indicator of water deprivation in the broiler rearing period that can be easily integrated into the quality control systems carried out at the slaughterhouse. This indicator should be informative of water deprivation during the on-farm period, as well as during the catching-to-slaughter interval. The accuracy of several parameters (e.g. skin turgor, capillary refill time, blood sodium, plasma chloride and creatinine concentrations) as indicator of different durations of water deprivation is investigated (Chapter3).

The second objective related to thirst is to evaluate a spontaneous water consumption test as an indicator for thirst in living broiler chickens. This indicator was previously validated in an experimental setting. The purpose of the current investigation is to apply the experimentally validated study on a larger scale and under practical circumstances. The influence of different factors such as stocking density, temperature and relative humidity on water consumption is evaluated. This test may form the basis of an animal-based indicator of thirst that could be included into on-farm welfare assessment schemes (Chapter 4).

The third objective is to assess automatic evaluation of footpad dermatitis which is also used as a measure for animal welfare of broiler chickens. Good scoring systems already exist for a trained assessor to assess footpad dermatitis. Because labor and time constraints limit the number of chickens per flock that can be assessed in large scale monitoring schemes, a prototype system for automatic scoring of footpad dermatitis at slaughter has recently been developed. In this study, the correspondence between the automatic scores and human expert scores given on farm and at slaughter are compared (Chapter 5).

CHAPTER 3:
DEHYDRATION INDICATORS FOR BROILER CHICKENS AT SLAUGHTER

Adapted from:

R. F. Vanderhasselt, S. Buijs, M. Sprenger, K. Goethals, H. Willemsen, L. Duchateau, and F. A. M. Tuyttens. 2013. Dehydration Indicators for Broiler Chickens at Slaughter. Poult. Sci. 92:612-619.

1. Abstract

Freedom of (prolonged) thirst is considered to be of paramount importance for animal welfare. This emotion normally results from dehydration, which can be measured using physiological indicators. Because no reliable physiological indicator for thirst was available for broilers, we aimed to identify such a measure in this study. This indicator would ideally be integrated into quality control systems in commercial slaughterhouses. In the first experiment, water deprivation was manipulated systematically by withdrawing water for different durations (total water withdrawal for 0 (control), 24, 36, or 48h, or a 10-day period with restricted access to water for two times 10 minutes per day). A significant decrease in absolute drained blood content and body weight occurred from 36h of total water deprivation onwards (both $P=0.03$), whilst long-term restricted access tended to decrease drained blood content ($P=0.05$). No effect of water deprivation or restriction on skin turgor was found. In the second experiment, water was withdrawn for 0 (control), 6, 12, 24 or 48h. Plasma chloride concentration was increased after 6h of water withdrawal, but did not rise further with longer withdrawal. If assessed at-slaughter, chloride will thus mainly reflect the catching-to-slaughter interval. In contrast, plasma creatinine and hematocrit levels showed a numerical decrease after 6h of water withdrawal, but rose again after prolonged withdrawal. Plasma creatinine values were significantly higher in 24h deprived birds than in 6h deprived birds ($P<0.01$), allowing for discernment between water withdrawal during catching and transport from dehydration that had occurred on the farm. Blood sodium concentrations and plasma osmolality showed a steady increment between 0 and 24h of water deprivation ($P<0.001$ and $P<0.001$ respectively), and may thus be used to assess the combined effects of water deprivation on farm and during the catching-to-slaughter interval. These findings may form the basis of an on-farm or at-slaughter test that could be included in integrated animal welfare assessment schemes.

Keywords: broiler, animal welfare, dehydration, water deprivation, assessment

2. Introduction

Freedom of (prolonged) thirst is generally considered to be of paramount importance for animal welfare (Brambell, 1965; Vanhonacker et al., 2008; Tuyttens et al., 2010). This state of discomfort cannot be assessed directly by physiological measures. However, water deprivation (i.e., a shortage of water intake compared to the physiological optimum) normally causes thirst, and physiological assessment of dehydration following water deprivation is thus likely to provide indirect information about thirst levels. In addition to its potential as a welfare indicator, water deprivation is also an indication of health problems (Manning et al., 2007) and decreased performance (Tabler, 2003; Viola et al., 2009). However, current methods for assessing water deprivation implemented within welfare monitoring schemes are not satisfactory (Sprenger et al., 2009). Such monitoring schemes usually evaluate absence of (prolonged) thirst by determining the number of birds per drinker place (e.g., Welfare Quality®, 2009). Although such resource-based indicators can often be assessed reliably and quickly, they do not assess water deprivation accurately or precisely, because birds that cannot reach or operate the drinkers will go unnoticed.

In an experimental setting, voluntary water uptake from an open drinker was shown to be a valid measure of thirst in broiler chickens, as it increased proportionally with the duration of water deprivation (Sprenger et al., 2009). This test may form the basis of an animal-based indicator of thirst that could be included into on-farm welfare assessment schemes. However, such tests are quite time consuming and give no information about the individual's level of thirst (Sprenger et al., 2009). Furthermore, measuring thirst on-farm will not give information about one of the most critical periods with regards to the risk of dehydration, i.e., during catching and transport to the slaughterhouse. In commercial practice, water is usually withdrawn just before the first bird of a flock is caught and crated for transport to the slaughterhouse. This depopulation process often takes several hours (crating rate of 1,000 to 1,300 broilers per hour per catcher (Delezie, 2006)). Water deprivation continues as the birds are transported to the slaughterhouse, a journey that may take several more hours. Upon arrival at the slaughterhouse, a variable amount of time passes before the birds are slaughtered (EFSA, 2011).

The objective of the present study was to identify a reliable and fast animal-based indicator of water deprivation in the broiler rearing period that can be easily integrated into the quality control systems carried out at the slaughterhouse. This indicator should be informative of on-farm water deprivation, as well as during the catching-to-slaughter interval. In literature, correlations between thirst/water deprivation and skin turgor (Laron, 1957; Laron et al., 1957), capillary refill time (EFSA, 2011), plasma osmolality (Butterworth et al., 2002), blood sodium, plasma chloride and creatinine concentrations (Knowles et al., 1995; Saito et al., 1998; Iheukwumere et al., 2003), blood volume and hematocrit levels (Zhou et al., 1998; Iheukwumere et al., 2003) have been described. In this study we investigate their accuracy as indicators of different periods of water deprivation, and discuss the feasibility of measuring them in a commercial setting.

3. Materials and methods

All procedures were approved by the ethics committee for animal experiments at the Institute for Agricultural and Fisheries Research (ILVO).

Dehydration is excessive loss of water from the body or from an organ or body part, as from illness or fluid deprivation (The American Heritage®, 2000). It can be caused by losing too much fluid, not drinking enough water or fluids, or both (Van Dale, 1990). We administered different degrees of dehydration by depriving birds of water during different duration periods (applying different water deprivation periods). Takei et al. (1988) already mentions that water deprivation provides a relatively natural method for producing thirst.

3.1 Experiment 1

Animals, housing and treatments

This experiment had four rounds. Within each round, 30 24-day-old Ross 308 broiler chicks (1:1 sex ratio) were housed in groups of six birds in littered floor pens of $2.2m^2$, except for the first round, which included only 18 birds. Ambient temperature varied between 18 and 22°C and an 18L:6D light schedule was used. A standard broiler diet was provided ad libitum. Until the start of the withdrawal treatments, water was also provided ad libitum.

Five treatments were applied. Chickens in the control treatment (0h) had continuous ad libitum access to water. In three other treatments, water was withdrawn by removing the drinker either 24, 36, or 48h prior to euthanasia. The fifth treatment consisted of long-term restricted access to water (10 days prior to euthanasia the drinker was removed, and was returned for 10 minutes twice per day). This treatment was designed to simulate some of the farm-dependent deprivation problems birds can experience such as inefficient use of drinking nipples due to leg disorders, high stocking density and disease (Houldcroft et al., 2008;

SCAHAW, 2000). At approximately 10 a.m. on day 40, all chickens were euthanized by cutting the jugular vein and carotid artery on one side of the neck. The carcasses were suspended upside down and allowed to bleed out.

Within each of the rounds, each treatment was randomly allocated to one pen, which is the experimental unit in our design. The exception to this was the first round, which included only a control group, and a 24h and a 48h water withdrawal group (in other words, no 36h water withdrawal or long-term water restriction group).

Measurements

Directly before euthanasia, body weight, capillary refill time and skin turgor were determined. Capillary refill time was measured by squeezing the wattle between the thumb and index finger for 10 seconds until the skin between the two fingers turned white, then recording the time needed for the color to return once pressure was released. To evaluate skin turgor, the skin of the birds' left thigh was taken between the thumb and index finger and lifted to a height of approximately 1cm, where it was kept for 10 seconds. 'Skin turgor time' was recorded as the interval between releasing the skin and the re-establishment of the previous skin condition.

During euthanasia, blood drained from the vessels of the neck of the inverted broilers was captured until blood flow was greatly reduced (after approximately 1.5 minutes), and subsequently weighed. This is not the most accurate method to determine blood volume, because an undetermined part of the blood remains in the body. However, birds are bled out in this manner during the slaughter process. This makes this measure easy and fast to perform in the slaughterhouse, which would allow an increased number of individuals to be assessed. We refer to this indicator as absolute drained blood content–and we also look at relative drained blood content (relative to body weight).

3.2 Experiment 2

Animals, housing and treatments

The second experiment had two rounds. Within each round, 30 37-day-old Ross 308 broiler chickens (1:1 sex ratio) were housed individually in pens of $0.5m^2$. Ambient temperature was kept at 21˚C, and a 20L:4D light schedule was applied. Water and standard broiler feed was available ad libitum until the respective starts of the experimental treatments. These broilers were randomly divided over five treatments: 0 (control), 6, 12, 24 and 48 h of water withdrawal, therefore bird is the experimental unit in this design. All treatments ended on day 39 between 12 a.m. and 3 p.m., when the birds were euthanized using a non-penetration captive bolt device ("CASH" Poultry Killer, Abato, Loon Op Zand, the Netherlands).

Measurements

Directly before euthanasia, blood was taken from the wing vein with a 25G needle and 2 ml syringe and collected in lithium-heparinized tubes. Immediately after blood collection, blood was aspirated in a heparinzed capillary tube (150 µl) and introduced into a blood gas analyzer (GEM Premier 3000, Instrumentation Laboratory, Zaventem, Belgium) for the determination of hematocrit (%) and sodium (mmol/l) levels. The remaining blood was centrifuged for 10 minutes at 3000 rpm, after which plasma was stored at -18°C for later analysis of osmolality, creatinine and chloride. Plasma osmolality (mosmol/kg) was measured with a vapor pressure osmometer 5500 (Wescor 5500 XRS, Prosan NV, Merelbeke, Belgium). Creatinine (µmol/l) was measured in the first round only, according to Helger's method (1974). Chloride concentrations (mmol/l) were measured using a Quantichrom™ Chloride Assay Kit (DICL-250, Gentaur, Brussels, Belgium).

Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS) version 9.3 for Windows (SAS Institute Inc., Cary, NC). Capillary refill, skin turgor time, absolute and relative drained blood content (relative to body weight) were analyzed using a mixed model (proc mixed). Round and pen were included as random effects, and treatment, sex and their interaction as categorical fixed effects. Observations on individual animals were used. Hematocrit, blood osmolality, blood sodium and plasma chloride concentrations were analyzed using the same mixed model, except that pen was omitted as a random effect (because broilers were housed individually). Plasma creatinine was measured in one round only, thus round was omitted from the model for this indicator. Statistical significance was evaluated at a significance level of 0.05. Fixed effects were tested with the traditional F-tests and degrees of freedom were predicted using the Satterthwaite formulas (Littel et al., 1996). Pairwise comparisons between treatments were tested at a total significance level of 0.05 using the Tukey-Kramer adjustment for multiple comparisons.

Receiver Operating Curves (ROC) were created for several indicators to show their sensitivity to 6 and 48h of water withdrawal. We decided to make ROC curves for plasma chloride and creatinine concentrations discerning between the control group and the 6h deprived group, and for blood sodium, plasma chloride and creatinine concentrations and hematocrit values discerning between the control group and the 48h deprived birds.

4. Results

4.1 Interaction and sex effect experiment 1 and 2

No significant interactions between treatment and sex effects were found for any of the investigated indicators in either of the experiments.

In contrast, some sex effects were found (Table 1). In the first experiment, males had a higher body weight and a higher absolute drained blood content than females. In the second experiment, males had higher sodium concentrations, plasma osmolality and hematocrit levels than females. The other indicators were not significantly affected by sex.

Table 1 Sex effect on the different physiological indicators investigated in experiment 1 and 2 as possible thirst indicator.

Indicator	**P-value sex**	**Least Squares Mean ± SEM**	
Experiment 1		Males	Females
Drained blood content, gram	<0.001	67.9 ± 3.9	55.8 ± 3.9
Body weight, gram	<0.001	2150 ± 59.9	1864 ± 59.9
Capillary refill, sec	0.788	1.39 ± 0.06	1.36 ± 0.06
Skin turgor, sec	0.675	0.77 ± 0.05	0.75 ± 0.05
Experiment 2		Males	Females
Sodium, mmol/l	<0.001	144 ± 1.2	137 ± 1.2
Osmolality, mosmol/l	0.004	297.7 ± 1.4	289.1 ± 1.4
Hematocrit, %	0.012	29 ± 1.7	28 ± 1.7
Chloride, mmol/l	0.185	331 ± 5.9	320 ± 5.9
Creatinine, µmol/l	0.801	43.7 ± 0.5	42.7 ± 0.5

4.2 Treatment effect experiment 1

Absolute drained blood content decreased steadily with increasing water withdrawal duration (P=0.009, Figure 1 A). Significant pair-wise differences (P<0.05) were found between control birds and birds deprived for 36 and 48h. Birds deprived for 36 and 48h tended (P<0.1) to have less blood than 24h deprived birds. Long-term restricted access also led to a tendency for decreased blood content compared to the control birds (P<0.1). Body weight decreased steadily with increasing water withdrawal duration (P=0.002, Figure 1 B). Significant pair-wise differences (P<0.05) were found between control birds and birds deprived for 36 (P=0.027) and 48h (P=0.003) and with those with long-term restricted access (P=0.006). The group of birds deprived of water for 24h showed higher body weights compared to the 48h deprived birds (P=0.027) and those with restricted water access (P=0.044).

Capillary refill time was influenced by the treatments (P=0.030, Figure 1 C). Capillary refill was significantly faster after long-term water restriction than after 36h of deprivation (P=0.021), with control birds having intermediate values. Capillary refill time tended to be faster in the broilers with long-term restricted access compared to birds subjected to 24h deprivation (P=0.075) and 48h deprivation (P=0.069).

Skin turgor (data not shown) was not affected significantly by the treatments (P=0.343).

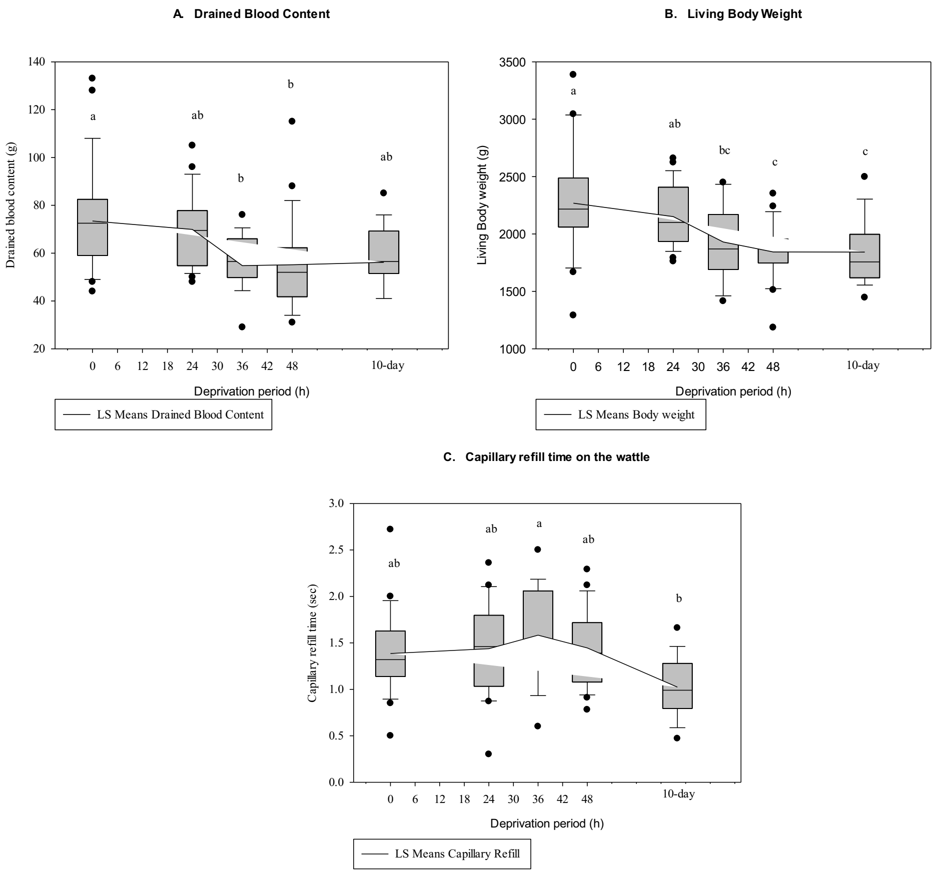


Figure 1. Box plots showing the response of broilers' absolute drained blood content (A), Living body weight (B) and wattle capillary refill time (C) to different periods of complete water withdrawal and to 10-day-long 20min/day water access. Least Squares Means without common superscript (a, b, c) differ significantly (P<0.05).

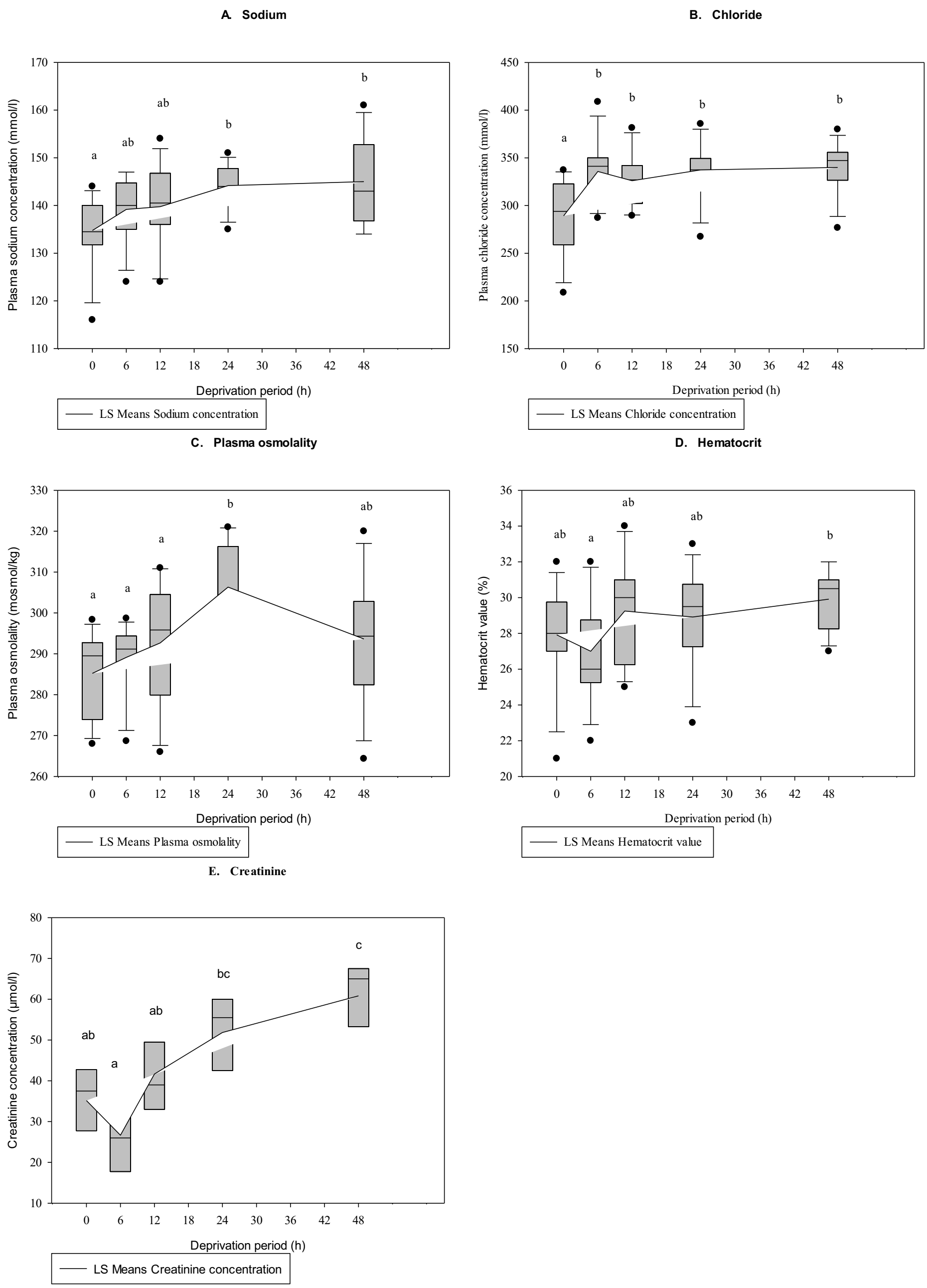


Figure 2. Broilers' physiological response to different durations of complete water withdrawal: whole blood sodium concentrations (A) and hematocrit values (D), plasma chloride (B) and creatinine (E) concentrations, and plasma osmolality (C). Least Squares Means without common superscript (a, b, c) differ significantly (p<0.05).

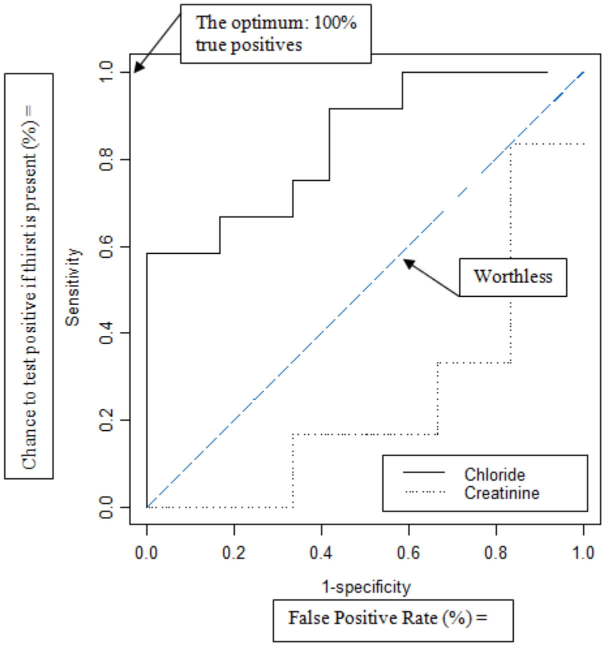
4.3 Treatment effect experiment 2

Duration of water withdrawal affected blood sodium concentrations and hematocrit values, plasma chloride and creatinine concentrations, and plasma osmolality ($P=0.001$, $P=0.017$, $P=0.001$, $P<0.001$, and $P<0.001$, respectively). Blood sodium concentration increased steadily with increasing duration of water withdrawal, becoming significantly higher than in the control birds after 24h of deprivation (Figure 2 A). Sodium concentration leveled off with longer water deprivation. Plasma chloride concentration increased significantly after 6 hours of water withdrawal, but did not rise any further with longer withdrawal (Figure 2 B). Plasma osmolality increased during the first 24h of water withdrawal, at which point values were significantly higher than those of the controls and 6h and 12h of water withdrawal (Figure 2 C). However, after 48h of withdrawal osmolality was decreased again. Hematocrit levels showed a somewhat erratic pattern: 6h of withdrawal led to numerically lower values than 48h of withdrawal, with all other treatments resulting in intermediate values (Figure 2 D). Plasma creatinine levels showed a non-significant decrease during the first 6 hours, but afterwards increased with longer water withdrawal periods. Creatinine levels were higher after the longest water withdrawal period than after the other periods. Birds deprived for 24h tended to have higher creatinine levels compared to the control ($P=0.085$) and 6h deprived birds ($P=0.004$) (Figure 2 E).

4.4 Receiver operating characteristic (ROC) curve analysis

Several potential parameters, suggested by the analysis described above were evaluated for their ability to indicate water deprivation in broilers using ROC curves (Figure 3). In a ROC curve, the sensitivity (true positives) is plotted against the false positives (1 - specificity) at different cut-off values. The most optimal cut-off value is in the upper left corner (Bradley, 1997): all samples detected are true positives. The area under the curve gives the performance of the test: 1 is a perfect test (no false positives) and 0.5 is a worthless test (same number of true positives as false positives). Figure 3 A illustrates the sensitivity of plasma chloride and creatinine to 6h of water deprivation. It shows that creatinine would be a bad indicator for a 6h water withdrawal period, as it stays close to the bisector. Chloride, on the other hand, would be much better. It would also be a good indicator for a 48h deprivation period (Figure 3 B). If no false positives would be allowed, nearly 60% of all 48h dehydrated birds would be detected. Creatinine concentration, however, comes out best for the detection of a 48h water withdrawal period. When no false positives would be allowed, 85% of the

A. 6h water withdrawal



B. 48h water withdrawal

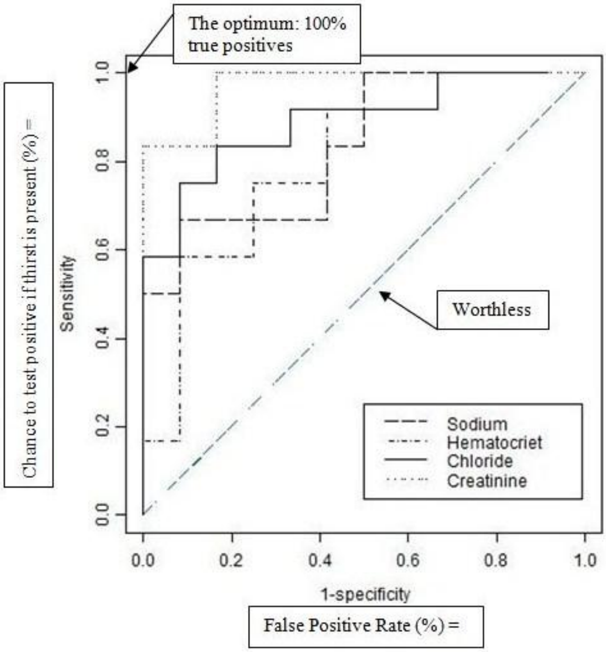


Figure 3 Receiver operating characteristic (ROC) curve for the detection of 6h water withdrawal (A), and 48h withdrawal (B). The closer the curve follows the left-hand border and then the top border of the ROC space, the more accurate the test. The closer the curve comes to the 45-degree diagonal of the ROC space, the less accurate the test.

deprived birds would be detected. A sensitivity of 100% for dehydration can be obtained with a 15% false positive rate. Sodium concentration in broilers' whole blood is presented third (Figure 3 B). Around 50% of the dehydrated birds would be detected.

5. Discussion and conclusion

Very little previous research has been conducted on the development and validation of indicators of dehydration when end-of-life broiler chickens are moved from the farm, transported and slaughtered. In this study, the validity of several potential water deprivation indicators was evaluated. Absolute drained blood content, body weight, capillary refill, sodium and chloride concentrations, plasma osmolality, and hematocrit and creatinine levels were found to respond to various stages of water deprivation.

The first category of indicators under discussion are those that responded to 6h water deprivation, but which did not show considerable changes after longer deprivation. Such indicators are of little value as at-slaughter indicators of water deprivation sustained on-farm. This is because the catching-to-slaughter interval (during which all birds are completely deprived of water) usually exceeds 6h, thus making it impossible to discern between birds that are only dehydrated due to this interval, and those that already suffered from on-farm water deprivation. Nevertheless, these indicators could be used to measure short-term on-farm water deprivation. In this study, chloride fits best into this category of indicators. However, this finding contrasts with previous research in which it took 72h for chloride concentrations to be significantly increased (Koike et al., 1983). This warrants further investigation. Furthermore, the sensitivity of chloride analysis as an indicator of short-term (6h) water deprivation was limited, as our model showed that less than 60% of deprived birds would be detected if no false positives are allowed. In animal welfare assessment schemes, it is important to minimize the number of 'false positives' (here non-deprived chickens that are classified as water deprived) because erroneous penalization may make farmers more reluctant to resolve the problem.

The second category is formed by those indicators that showed opposite reactions to short (6h) and medium or long water withdrawal (24, 36 or 48h). Such indicators can discern between water deprivation sustained on-farm and caused by the catching-to-slaughter interval. Our study indicates that plasma creatinine values showed the greatest potential to do so. Increases in broilers' creatinine levels caused by medium and long term dehydration had not been evaluated prior to this study, but a study in pigeons (Lumeij, 1987) corroborates our findings. Also, a study from Iheukwumere et al. (2003) observed higher creatinine levels in broilers given restricted access to water. In addition, the sensitivity of the creatinine test was high, detecting over 80% of 48h dehydrated birds if no false positives are allowed, and all if 20% false positives were allowed. Hematocrit followed approximately the same pattern as creatinine during the first stages of water withdrawal, it showed no further increase when withdrawal exceeded 12h. Also the ROC curve of hematocrit was not good (low sensitivity when specificity was high). Therefore, creatinine analysis would be preferred to hematocrit analysis. The slightly erratic response of hematocrit to water withdrawal is in line with previous research (Zhou et al., 1998).

The third category consists of indicators which, when applied at-slaughter, would assess the combination of on-farm deprivation and catching-to-slaughter deprivation. Whole blood sodium concentration showed an approximately steady increase during the first 24h of water withdrawal and remained stable afterwards. This is supported by many other studies in layer and broiler chickens (Chamblee et al., 1982; Koike et al. 1983; Arad et al., 1985; Chamblee et al., 1988; Robinzon et al., 1990; Swayne et al., 1991; Knowles et al., 1995). Therefore, sodium concentration can be used to detect 'medium term' (24h) water deprivation, although a non-significant increase occurred already after 6h in our study. The sensitivity of this test is limited, however, as only 70% of dehydrated birds would be detected even when 10% false positives are allowed. Plasma osmolality also showed a clear increase after 24h of water deprivation, in line with Koike et al. (1983). But plasma osmolality values decreased again between 24h and 48h of water withdrawal. This may be because prolonged withdrawal causes decreased feed intake (Koike et al., 1983), which in turn results in decreased plasma osmolality (Knowles et al., 1995). The validity of the 48h decrease found in our study can be questioned, as it is not supported by previous studies using deprivation periods of 48h (Koike et al., 1983; Arad et al., 1985) or longer (Butterworth et al., 2002; Swayne et al., 1991). Absolute drained blood content seemed most suited to detect long (≥36h) water deprivation. There was no distinct drop in drained blood content before 24h of water withdrawal, in line with previous results in layers (Koike et al., 1983), but blood content more or less stabilized after 36h of water withdrawal. However, considerable within-treatment variation was observed, which resulted in low sensitivity.

The last category of indicators consisted of those that could be used on-farm to detect birds that will only drink occasionally. This can occur when severely lame birds stand up to drink only when very thirsty, or when small birds can only access drinkers at specific times (e.g., when other birds lay down near the drinker and can be used as a stepladder, or when water is spilled). This situation was simulated by the long-term restricted access treatment, for which only drained blood content, capillary refill, skin turgor and body weight were measured. Neither indicator was significantly affected by long-term restricted access (as compared to controls), although absolute drained blood content and capillary refill were numerically decreased after applying such a treatment.

Feasibility of application under commercial circumstances is of great importance for welfare assessment in non-experimental settings. Blood sampling could be carried out rapidly in the slaughterhouse, but physiological blood indicators that require chemical analysis in the laboratory may be too time-consuming, impractical or costly for large-scale monitoring applications. However, these physiological indicators show good potential and therefore it would be valuable to develop easily, not too costly methods for measuring them at the slaughter line.

In conclusion, these findings illustrate the potential of animal-based measures to assess different stages of dehydration in broilers. Such tests may form the basis of an on-farm or at-slaughter test that could be included in integrated animal welfare assessment schemes. Of all tested indicators, plasma chloride concentration may be most suitable to detect the effects of transport. The best indicators of medium-term water deprivation were creatinine and sodium. Measuring protocols for more easily applied indicators (blood content, capillary refill) should be optimized if they are to be used to evaluate dehydration, as their sensitivity is currently poor.

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CHAPTER 4:

AN ANIMAL-BASED THIRST INDICATOR FOR BROILER CHICKENS ON FARM

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1. Abstract

Animal-based measures of thirst are currently absent from animal welfare monitoring schemes due to the lack of a well-validated indicator applicable for on-farm use. In the present study an on-farm test based on spontaneous water consumption from an unfamiliar open drinker was validated in a (semi-)commercial setting. To investigate the effect of thirst on water consumption, we subjected four flocks of 1,500 broilers to either 0 or 12h of water deprivation and subsequently measured the amount of water that small subgroups consumed after the deprivation period (first experiment). Broilers that were water deprived prior to the test drank more than control broilers ($P<0.001$). In a second experiment, a similar test was performed using 20 commercial broiler flocks in Belgium and Brazil. After a pre-treatment water consumption test, the birds were subjected to 0 or 6h of water deprivation, and a post-treatment water consumption test was conducted. Only in Brazil, deprived birds drank significantly more than controls in the post-treatment water consumption test ($P<0.001$). A tendency for a difference was found in Belgium ($P=0.083$). Pre- and post-treatment water consumption was higher in Brazil than in Belgium ($P<0.001$). Temperature, stocking density and wind velocity influenced the pre-treatment water consumption in Brazil, but not in Belgium. These results indicate that the water consumption test is sufficiently sensitive to discriminate between control and 12h deprived flocks, and in Brazil even between control and 6h deprived birds. The location of the test within the house did not affect the amount of water consumed in either experiment, suggesting that this variable does not have to be standardized. However, the data from Brazil indicate that the amount of water consumed by broilers able to drink freely for a long period depended on indoor climatic variables and possibly genotype. This suggests that these variables need to be considered when interpreting the test outcome in terms of the thirst level experienced by the broilers.

Keywords: broiler, animal welfare, dehydration, water deprivation, assessment

2. Introduction

Thirst, the subjective perception that motivates an animal to drink, is considered to have a major impact on animal welfare (Brambell, 1965; Vanhonacker et al., 2008; Tuyttens et al., 2010). Thirst is activated by several factors related to water loss or dehydration, e.g., decreased bodily fluids, increased blood osmolality, or changes in the activity of specific dipsogenic hormones (McKinley et al., 2004). Several of these physiological changes have shown potential as indicators of thirst (Chapter 3). However, physiological mechanisms buffer fluctuations in blood parameters, and changes will therefore only be detected when such buffering mechanisms fail as a result of severe dehydration (Sprenger et al., 2009). Furthermore, the chemical analyses needed for such indicators are often too time-consuming, impractical and costly to incorporate these into large-scale monitoring schemes (Chapter 3).

Current methods for assessing thirst within poultry welfare monitoring schemes are not satisfactory (Sprenger et al., 2009). Such monitoring schemes usually evaluate thirst by determining the number of birds per drinker place and/or the drinking water quality (e.g., Welfare Quality®, Belplume specifications, RSPCA welfare standards). Although such resource-based indicators can often be assessed reliably and quickly, they do not assess thirst accurately or precisely. For example birds that cannot reach or operate the drinkers are likely to go unnoticed. Resource-based indicators represent risk factors rather than indicators of the true welfare status. Animal-based measures are thus better suited to evaluate animal welfare (Whay et al., 2003), although their validity should be established before they can be incorporated into a welfare evaluation scheme.

A test based on recording voluntary water uptake from a nearby and easy-to-access open drinker for 1-2h has recently been shown to be a valid animal-based measure of thirst in broiler chickens kept in an experimental setting, as the voluntary water uptake increased proportionally with the duration of water deprivation (Sprenger et al., 2009). This test is referred to below as a “water consumption test”.

The purpose of the current study was to assess the validity of this water consumption test when applied to large groups of birds in a commercial setting. To do so, we first performed a test on a semi-commercial farm (poultry research institute doing applied research in which commercial husbandry conditions are approximated) (experiment 1). Thirst was induced by depriving groups of birds from water for 12h, and subsequently allowing the birds to drink. Their intake was compared to that of birds that had not been deprived prior to testing. This test was carried out at different locations within the housing unit to verify whether location influenced the test outcome. If so, the test would need to be strictly standardized when used on-farm.

Subsequently, a similar but shortened thirst test (in which water consumption was monitored for 90 instead of 120 min) was implemented on several commercial farms for further validation and sensitivity testing (experiment 2). Water consumption was measured before and after 0 vs. 6h water deprivation treatments. Pre-treatment measurements of birds were used to assess how various animal, farm and environmental factors (e.g. body weight, stocking density, temperature and relative humidity) influence water consumption without water deprivation. Post-treatment measurements of control birds were also used to assess how such factors influence the water intake of birds assumed to be free of thirst (controls had easy access to water in the 6h period prior to the test). The effect of the deprivation treatment on post-treatment consumption was evaluated to test whether the thirst test was sufficiently sensitive to detect a relatively short duration of water deprivation in a commercial setting. Finally, the correspondence between this animal-based thirst indicator and the resource-based thirst indicator used in the Welfare Quality® (Welfare Quality®, 2009) broiler assessment protocol was evaluated.

3. Materials and methods

3.1 Experiment 1: semi-commercial setting

Animal, housing and treatment

Broilers (Ross 308, 1:1 sex ratio) were housed in four flocks of 1,500 birds per pen (72m²) and kept under conditions typical for commercial poultry farms in Belgium (kept on an experimental farm, Proefbedrijf Pluimveehouderij, Geel, Belgium). A standard broiler diet was provided ad libitum. Until the start of the withdrawal treatments, water was provided ad libitum.

Within each of the two rounds that were carried out, two flocks were allocated to each of the two treatments. Chickens in the control treatment (0h) had continuous ad libitum access to water prior to the water consumption test. In the other treatment (12h), water was withdrawn by raising the drinker line out of reach at 10 p.m. when the chickens were 34 days old. Twelve hours later, three groups of five focal birds per flock were separated from the rest of their flock using a temporary enclosure (a 1 m² enclosure that allowed visual and auditory contact with the remainder of the flock). These three groups were caught and enclosed within three different locations within the pen: one group in the far left corner, one in the centre, and one in the nearest right corner from the entrance. An unfamiliar open drinker (with a height of 7 cm to make sure that the water was clearly visible to the birds) was placed in each of the temporary enclosures, and the amount of water consumed from these drinkers was measured during 120 min (as described in detail in Sprenger et al., 2009).

This procedure was repeated 48 hours later, but the flocks that were previously deprived now served as controls, whereas the previous controls were now deprived for 12h prior to testing. None of the focal birds used in the first test (which were marked after the test) were used as focal birds in this second test.

3.2 Experiment 2: commercial setting

Animals and housing

Ten commercial broiler farms in the northern area of Belgium (Flanders) and ten farms in the south of Brazil (region of Passo Fundo, State of Rio Grande do Sul) were visited one or two days before slaughter. Farm visits in Belgium and Brazil were carried out in May and October 2011, respectively. The farms visited in Brazil all produced broilers destined for the European market. Flock records were used to obtain the number of birds in the house, their age and breed, and the cumulative mortality and culling percentage up to the day of the researcher's visit. Temperature, relative humidity and wind velocity were measured in the house at bird height using a portable device (testo 410-2, Testo NV/SA, Ternat, Belgium). The total house area (in m²), heating type (wood, gas or hot air gun), drinker type (nipple, cup or bell drinker), litter type (wood shavings, straw or chopped straw) and the average weight of the birds at the time of the visit were either recorded upon entry to the house or were provided by the farmer. From this information, the stocking density (kg/m²), the total mortality percentage (sum of natural mortality and culling percentage), the water vapor density, the Welfare Quality® score for the criterion absence of thirst and the adjusted drinker ratio were calculated. Water vapor density (which reflects the water vapor gradient between the bird and its environment and therefore the potential for evaporative heat exchange (Mitchell et al., 1998)) was generated from the temperature and relative humidity using an algorithm provided by M. Mitchell (Rural Research, Education & Consulting, Midlothian, UK, personal communication). The Welfare Quality® score for the "absence of thirst" criterion was calculated through the integration method provided by Welfare Quality® (Welfare Quality®, 2009). The true drinker ratio is adjusted in order to be able to compare the different drinker ratios between the poultry houses, which use different drinker types. The adjusted drinker ratio was calculated by dividing the actual drinker ratio (number of birds over number of drinkers in the poultry house) with the recommended number of birds for that specific type of drinker according to Welfare Quality® (Welfare Quality®, 2009): 1 nipple drinker per10 birds, 1 bell drinker per 100 birds and 1 cup drinker per 28 birds. A value > 1 means there were more drinkers in the poultry house compared to recommended. A value < 1 indicates there are fewer drinkers available compared to recommended by Welfare Quality® (Welfare Quality®, 2009). Table 1 provides basic management information on the farms visited in Belgium and Brazil.

All Belgian farms reared mixed-gender Ross flocks (as is typical for the Belgian broiler production) in closed poultry houses. All Brazilian farms reared male Cobb birds for the same slaughterhouse in open-sided poultry houses with curtains at both walls.

Water consumption test

In contrast to the protocol described above for the semi-commercial setting, water consumption was now measured before as well as after the deprivation treatments (0h and 6h) had been applied. In each poultry house, four groups of five birds were separated from the flock in temporary enclosures at different locations within the house (two enclosures in the centre of the house and two against opposite walls). Only one house per farms was included in the experiment. Birds were unable to access the normal drinker and feeder lines from within the temporary enclosures.

Pre-treatment water consumption test. After the groups were enclosed, each group received an unfamiliar open drinker and their water consumption was measured. Compared to experiment 1, the duration of recording water consumption was reduced from 120 to 90 min, with the intention of improving the practical feasibility of the test.

Post-treatment water consumption test. After the pre-treatment test one group enclosed near the wall and one enclosed in the centre of the house were randomly allocated to the 6h water deprivation treatment. Their drinker was removed from their enclosure. For the other two groups (the experimental controls) the drinker remained in their enclosure (0h water deprivation treatment). Six hours later all groups (6h as well as 0h) were provided with a new drinker. Water consumption was again measured during 90 minutes.

Table 1 Information of the farms visited in Belgium (BE) and Brazil (BR): the age and body weight of the birds, house area, applied stocking density at the day of the visit, Welfare Quality® score for the "absence of thirst" criterion (WQ Thirst score), the adjusted drinker ratio, the drinker and litter type, the total mortality until the day of the visit and temperature (Temp), relative humidity (RH), water vapor density (WVD) and wind velocity in the poultry house at the time of the visit, and the pre-treatment and control birds post-treatment water consumption.

Country	Age (days)	Body weight (g)	House area (m^2)	Stocking density (kg/m^2)	Adjusted drinker ratio	Drinker type	Total mortality (%)	Litter type	Temp (°C)	Wind velocity (m/s)	RH (%)	WVD* kg/m^3	WQ Thirst score	Pre- Treatment water consumption (g/bird/90min)	Control's Post- Treatment water consumption (g/bird/90min)
BE	39	2500	700	46	1.9	bell	3.2	Chopped straw	26	0.3	64	16.0	86.8	53.5	98.5
BE	36	2100	950	43	1.0	cup	5.6	Chopped straw	25	0.4	44	10.0	80.2	82.8	22.5
BE	40	2227	845	41	1.4	cup	8.7	Straw	24	0.2	62	13.6	38.6	2.0	19.0
BE	40	2488	700	39	0.9	nipple	2.8	Straw	27	0.2	60	15.5	34	31.3	72.5
BE	41	2566	1700	41	0.8	nipple	3.0	Chopped straw	28	0.2	41	11.2	46.3	17.8	54.5
BE	36	2200	1400	39	1.2	nipple	3.1	Wood shavings	28	21.6	35	9.3	48.2	55.5	124.0
BE	37	2160	1800	38	1.5	nipple	6.3	Chopped straw	27	0.3	49	13.0	67.8	21.5	57.0
BE	40	2450	1470	40	1.3	nipple	1.3	Chopped straw	26	0.1	50	12.2	40.6	93.5	26.5
BE	38	2300	1260	37	0.8	nipple	2.1	Wood shavings	25	0.3	68	16.1	30.5	2.0	20.5
BE	39	2300	1300	35	1.3	nipple	4.8	Wood shavings	25	0.5	53	12.2	16.3	33.0	8.0
BR	40	2550	1200	27	0.6	nipple	6.6	Wood shavings	26	0.5	56	13.7	97.7	183.5	60.5
BR	42	2600	1200	27	0.7	nipple	4.3	Wood shavings	27	0.4	65	16.8	91.1	94.3	68.0
BR	42	2860	1200	31	0.8	nipple	4.2	Wood shavings	31	1.2	32	10.3	83.9	233.3	118.0
BR	40	2550	1200	29	0.5	nipple	5.2	Wood shavings	29	0.8	49	13.9	100	363.8	68.0
BR	40	2450	1200	29	0.8	nipple	5.5	Wood shavings	32	0.7	33	11.2	81.7	278.3	140.0
BR	35	2160	600	22	0.8	nipple	2.9	Wood shavings	28	8.3	40	10.6	86.5	21.3	157.0
BR	44	2950	1820	30	0.5	nipple	6.8	Wood shavings	30	1.0	34	10.5	100	148.8	79.5
BR	37	2150	1200	22	0.8	nipple	4.0	Wood shavings	24	0.3	74	16.5	86.3	108.8	73.0
BR	42	2800	1200	31	0.7	nipple	4.2	Wood shavings	28	0.7	47	12.5	91.4	250.0	49.0
BR	40	2630	1200	27	0.8	nipple	6.9	Wood shavings	25	0.6	59	13.2	87.3	108.0	23.0

* Water vapor density (WVD)

3.1 Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS) version 9.3 for Windows (SAS Institute Inc., Cary, NC). Water consumption was analyzed using the mixed model with pen and location nested in pen as random effects in the first experiment, and farm and location nested in farm as random effects in the second experiment. For the first study, location, age and treatment were introduced as categorical fixed effects, whereas in the second experiment, country, treatment, location, breed, litter type, heating type and drinker type were introduced as categorical fixed effects. In the second experiment, three sets of water consumption data were analyzed: 1) pre-treatment water consumption test; 2) post-treatment water consumption test for all birds and 3) post-treatment water consumption test for control birds only. Finally, we evaluated the effect of the covariables on pre-treatment water consumption and on control birds' post-treatment water consumption. Statistical significance was evaluated at a significance level of 0.05. Fixed effects were tested using the F-test at a significance level of 5%. Pairwise comparisons between factor levels were tested at a global significance level of 5% using the Tukey-adjustment technique for multiple comparisons. The Pearson correlation between the pre-treatment water consumption and the Welfare Quality® score for absence of thirst was calculated per country.

4. Results

4.1 Experiment 1: semi-commercial setting

Birds drank more during the 120 min water consumption test when they had been deprived for 12h prior to testing ($P < 0.001$, Figure 1). Water consumption did not differ significantly between birds of 35 days and 37 days of age ($P = 0.089$). Test location in the poultry house was not found to affect consumption ($P = 0.585$).

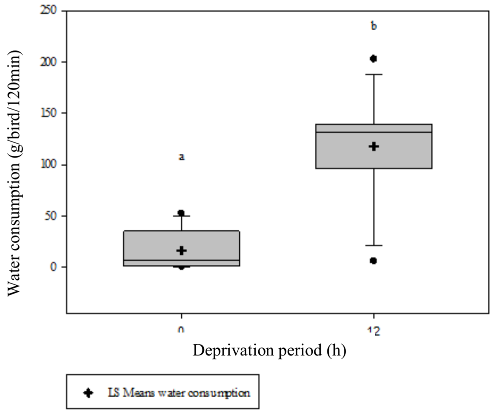

Figure 1 Box plot of the water consumed by broilers following a water deprivation period of 0 versus 12h (Experiment 1). Least Squares Means without common superscript (a, b) differ significantly (p < 0.05).

4.2 Experiment 2: commercial setting

For the second experiment, we first compared the post-treatment water consumption test between Belgium and Brazil. As many variables were highly correlated with country, we investigated the effects of the variables on water consumption for the Belgian and Brazilian data separately.

Water consumption in Belgium and Brazil.

Pre-treatment water consumption was higher in Brazil than in Belgium ($P < 0.001$, Figure 2), but was not significantly affected by test location or by interactions with location. Brazilian birds drank much more during the post-treatment water consumption test when previously deprived than when non-deprived ($P < 0.001$), whereas a much smaller and non-significant increase in water consumption was found in Belgian deprived birds ($P = 0.083$). No difference was found between the post-treatment water consumption of the Brazilian control birds compared to the Belgian control birds ($P = 0.160$), whereas Brazilian deprived birds drank more than their Belgian deprived counterparts ($P < 0.001$, Figure 3). Test location and possible interaction terms with location showed no effect on the post-treatment water consumption of the birds ($P > 0.100$). The Welfare Quality® scores for absence of thirst were not correlated with the water consumption of Belgian ($r = -0.10$, $P = 0.55$) or Brazilian ($r = 0.20$, $P = 0.23$) birds.

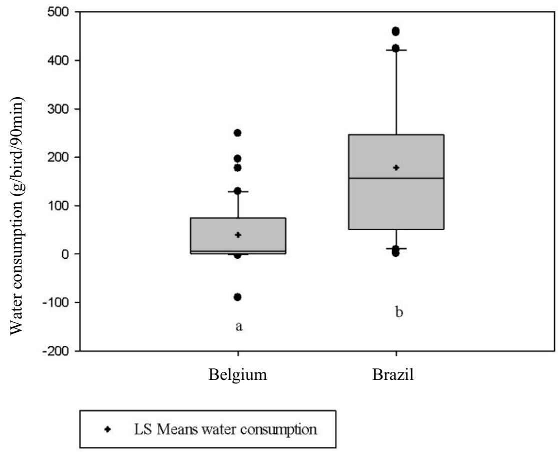

Figure 2 Box plot of the pre-treatment water consumption of broilers in Belgium and Brazil (experiment 2). Least Squares Means without common superscript (a, b) differ significantly ($p < 0.05$).

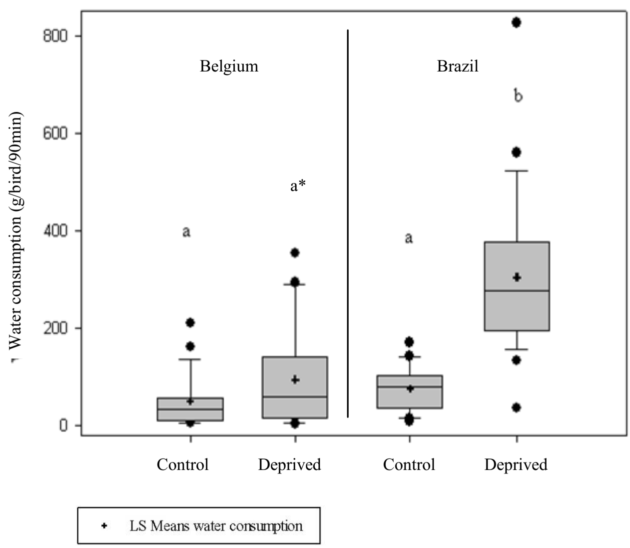

Figure 3 Box plot of the post-treatment water consumption of the control (0h deprivation treatment) and deprived (6h deprivation treatment) broilers in Belgium (left side) and Brazil (right side) (experiment 2). Least Squares Means without common superscript (a, b) differ significantly ($p < 0.05$ and * $p < 0.10$).

Effect of the covariables on water consumption in Belgium and Brazil.

Farms in Belgium and Brazil differed substantially (Table 1). The Belgian farms only housed Ross, birds whereas the Brazilian farms all housed Cobb broilers. All Brazilian farms used wood shavings as litter, whereas Belgian farms either used wood shavings, straw or chopped straw. In Belgium all houses were heated by hot air guns, whereas Brazilian houses were heated with wood, gas, or both wood and gas. One Belgian house was equipped with bell drinkers and two Belgian houses were equipped with cup drinkers. All other houses were equipped with nipple drinkers. Because of these differences, the effect of different covariables on pre-treatment and post-treatment water consumption is studied separately in the two countries. Within Belgium, no effect of drinker type (P = 0.404) and litter type (P = 0.407) was observed on the test outcome. Heating type did not affect water consumption in Brazil (P = 0.196).

In Figure 4, the difference (and the 95% confidence interval) in water consumption between two relevant levels of the particular covariate is presented. Whenever the 95% confidence interval contains zero, the two levels do not differ significantly from each other.

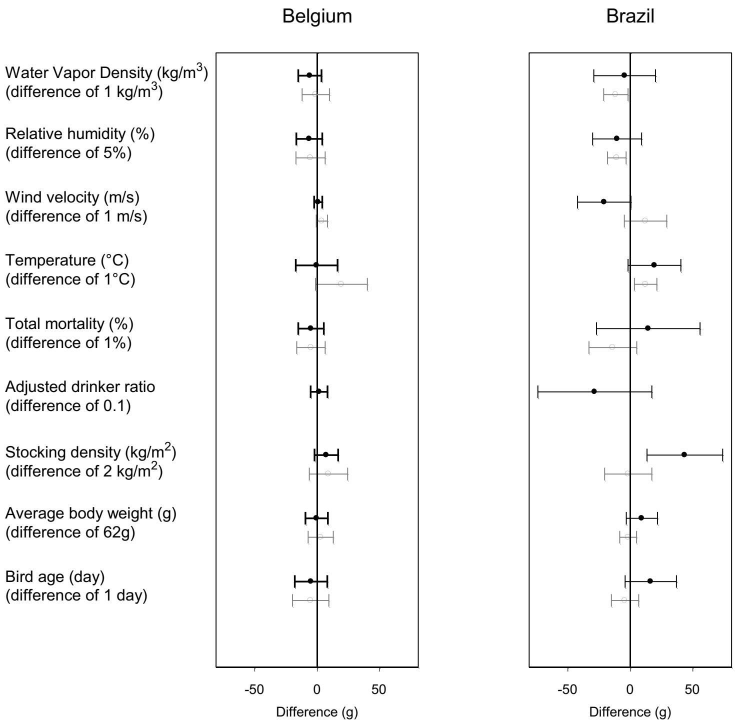

Figure 4 The difference in water consumption (95% confidence interval) for two relevant levels for particular covariates is presented for pre-treatment water consumption (black line) and for the control's post-treatment water consumption (grey line) in Belgium (left panel) and Brazil (right panel).

In Belgium, none of the variables analyzed affected the pre-treatment consumption test (all P > 0.136). Post-treatment water consumption of the Belgian control birds tended to increase with temperature (P = 0.084). In Brazil, pre-treatment water consumption did not change significantly with temperature (P = 0.089), but post-treatment consumption of control birds increased significantly with temperature (P = 0.025). In Brazil, pre-treatment water consumption increased with increasing stocking density (P = 0.011) and tended to decrease with wind velocity (P = 0.071). Brazilian control birds consumed less water in the post-treatment test when relative humidity and water vapor density were higher (P = 0.020 and P = 0.048, respectively). Adjusted drinker ratio did not affect pre-treatment water consumption in Belgium (P = 0.541), nor in Brazil (P = 0.237).

5. Discussion

Little research has been conducted on the development and validation of indicators for on-farm evaluation of thirst of broiler chickens. In this study, a water consumption test previously validated in an experimental setting (Sprenger et al., 2009) was evaluated under (semi-) commercial circumstances on Belgian and Brazilian farms. The water consumption test was found to respond to a water deprivation period of 12 and even 6 hours, supporting its validity as a thirst indicator appropriate for a commercial setting. Location within the house was not found to influence the test outcome of the present study, indicating that strict standardization of this factor does not seem necessary. The drinker type used in the poultry house and the birds' age and average body weight were not found to influence the post-treatment water consumption test, indicating that strict standardization of these factors does not seem necessary either.

The effect of country depended on the amount of access to water prior to testing. There was no significant difference in water intake between Brazilian and Belgian broilers when tested after 6 hours of easy access to water (i.e., penning near an open drinker), but in absolute values Brazilian birds drank more. Brazilian broilers drank significantly more than Belgian broilers prior to the deprivation treatments. Brazilian broilers also showed a greater increase in their water consumption after 6h deprivation than Belgian broilers did. This indicates that Brazilian broilers became thirstier when not given easy access and drank more when easy access was provided. This could also indicate that they had greater difficulties in securing access to water under commercial circumstances (e.g. due to differences in the birds' locomotor ability), or that they needed to drink more water to maintain body fluid homeostasis, owing to differences in genotype, climate or management. Because country cannot be isolated from several other variables (e.g. breed, litter type, heating type, drinker type), this effect on the test outcome can hardly be claimed as a true country effect. It seems plausible that part of the explanation lies in the breeds used in both countries. Belgian farms reared Ross broilers, whereas the Brazilian farms reared Cobb broilers. Management manuals of both breeds reveal a higher water maintenance need for Cobb broilers compared to Ross birds. The recommended water (in liters) to feed (in kg) ratio at 20°C is 2:1 for Cobb and 1.6:1 for Ross broilers (Ross, 2009; Cobb, 2012). Differences in leg weakness among commercial genotypes have been described in previous research (Kestin et al., 1999; Almeida et al., 2010). Ross birds have been described to have better leg health than Cobb birds and may thus be better equipped to move towards normal drinkers and to operate these, resulting in a decreased need for water intake in the water consumption test.

The Brazilian subset of the data showed a significant or almost significant effect of temperature, wind velocity and stocking density when the test was carried out without prior deprivation (i.e., as it would be used in practice to assess welfare on-farm). These effects were all expected. Increasing stocking densities will increase the environmental temperatures at bird level within the poultry houses. Increased temperature will cause panting, which results in moisture loss and thus a need for increased water intake (Fairchild et al., 2009), whereas increased wind velocity will cool birds down, decreasing the need to pant. The effect of stocking density might be explained by the greater difficulty birds may have to reach the drinkers when they are stocked more densely (e.g., because they have to push their way through more birds to reach drinkers). This may result in greater thirst in crowded flocks than non-crowded flocks. However, in the Belgian subset no such effects were shown, which is surprising since stocking density was higher and the number of drinkers lower. This suggests that for birds which are more prone to dehydration, a more pronounced effect of climatic variables, and possibly to a lesser extent management factors, can be seen. These variables should therefore be seen as risk factors for thirst. If such a test were to be performed within a welfare assessment scheme (i.e. without applying water deprivation prior to testing) the interpretation of the amount of water consumed during the test (in terms of the amount of thirst experienced by the birds) may have to be adapted according to the breed and the climate inside the housing unit. The data from the post-treatment test of the control birds show that birds with easy access to water increase their water intake when kept in a warm environment with a low humidity (probably to maintain body fluid homeostasis). However, as long as birds in such environments can readily access water, their motivation to drink will not be thwarted to such an extent that it becomes a welfare problem. This means that the threshold value for the amount of water consumed during the test ought to account for these confounding climatic variables. If the water consumption test is to be used to compare thirst levels in flocks of various lines and kept under very different climatic conditions, further work is recommended to clarify the relation between the management and climatic variables and the water intake required for homeostasis.

In existing broiler welfare evaluation schemes, freedom from thirst is assessed by a resource-based indicator such as the number of animals per drinker, checking possible leaks in the drinker line and checking drinker alarms (Sprenger et al., 2009). Welfare Quality® calculates a welfare score for thirst according to an index expressing the percentage of compliance with the recommended number of drinking places. If this compliance is higher than 90%, the welfare score approximates the maximum score of 100 for the absence of prolonged thirst criterion. If this compliance is 40%, the welfare score approximates half of the maximum score, a score of 50 for the criterion “absence of prolonged thirst”. In Belgium, all poultry houses had a score for absence of prolonged thirst lower than 90. In Brazil, six out of ten farms had a score below 90. However, all six of these Brazilian farms had a score higher than 80, indicating high welfare standard for thirst. Seven out of ten Belgian farms had a score for absence of prolonged thirst below 50, indicating a just acceptable level of thirst (Table 1). Broilers in these seven Belgian farms with a low score (< 50) did not drink more during the pre-treatment water consumption test compared to the other farms. The pre-treatment water consumption test of the Belgian farms with a high score (> 80) did not consume less water during the pre-treatment water consumption test compared to the other farms, in fact, their water consumption was rather high. Within a country, the Welfare Quality® criterion score for absence of thirst and the pre-treatment water consumption test were not correlated. These examples illustrate the more general discrepancy between the outcome of the animal-based water consumption test and the resource-based Welfare Quality® score.

When taking both experiments into consideration, it appears that the water consumption test as performed in the present study is sufficiently sensitive to discriminate between flocks that have not been water-deprived from flocks that have been deprived for 12h. A deprivation period of 6h was detected for the Brazilian farms, but was not detected for the Belgian farms according to the statistical norm of a significance level of 5%. Possibly the sensitivity of the test could be increased by extending the duration of the water consumption test or by increasing the number of broilers per flock subjected to the test.

The findings indicate that voluntary water consumption from an open drinker shows great promise for an on-farm test of thirst that could be included in broiler welfare assessment schemes. The test design should be optimized further to account for the effect of confounding variables on the interpretation of the test and to determine the optimal number of birds to be tested per flock.

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CHAPTER 5:

AUTOMATED ASSESSMENT OF FOOTPAD DERMATITIS IN BROILER CHICKENS AT THE SLAUGHTER LINE: EVALUATION AND CORRESPONDENCE WITH HUMAN EXPERT SCORES

Adapted from:

R. F. Vanderhasselt, M. Sprenger, L. Duchateau, and F. A. M. Tuyttens. 2013. Automated Assessment of Footpad Dermatitis in Broiler Chickens at the Slaughter line: Evaluation and Correspondence with Human Expert Scores. Poultry Sci. 92:12-18.

1. Abstract

Footpad dermatitis is increasingly used as an indicator of decreased broiler welfare, and automation of dermatitis monitoring potentially reduces the effort needed to monitor commercial flocks. In this study we evaluated a prototype system for the automatic assessment of footpad dermatitis in broiler chickens, by comparing the automatic assessment with a human expert assessment. The expert aimed at selecting two times (different period) 20 broilers per footpad dermatitis category (5 categories in total), from two different flocks of 38-day-old broilers on an experimental farm. Two days later these broilers were transported to the slaughterhouse, where footpad dermatitis was assessed by the automatic system. Subsequently the footpads were re-assessed by the same expert that had selected the birds. Automatic scores were only weakly correlated with scores given by the expert on-farm ($r = 0.54$) and at the slaughterhouse ($r=0.59$). Manual evaluation of the photographs on which the automatic system based its scores revealed several errors. For 41.1% of the birds the automatic system assessed only one of the footpads, whilst for 15.2% neither footpad was assessed. For 49.4% of the birds, scores were based on partially incorrectly identified areas. When data from such incomplete and obviously incorrect assessments were discarded, stronger correlations between automatic and expert scores were found ($r = 0.68$ and $r = 0.74$ for expert scores given on-farm and at-slaughter, respectively). Footpads that were missed by the automatic system were more likely to receive a high expert score at slaughter ($P = 0.02$). However, average flock scores did not differ greatly between automatic and expert scores. The prototype system for automatic dermatitis assessment needs to be improved on several points if it is to replace expert assessment of footpad dermatitis.

Key words: broiler, animal welfare, dermatitis, automation

2. Introduction

Footpad dermatitis, which affects the plantar region of the feet, is the most common type of contact dermatitis in broiler chickens. It is a type of lesion which could heal, but which rarely does under commercial conditions (Martland, 1985; Berg, 2004). Dermatitis may cause pain and deserves consideration in the context of animal welfare (Berg, 2004). Furthermore, footpad dermatitis has financial implications for producers (Dowsland, 2008).

It is widely accepted that footpad dermatitis is related to litter quality, and more specifically the amount of moisture, ammonia and other chemical substances it contains (Berg, 2004; Eichner et al., 2007; Allain et al., 2010). Many other factors (e.g., breed, diet, stocking density, drinker system, nutrition and enteric health) can also influence the occurrence of footpad lesions (Mayne, 2005; Eichner et al., 2007; Musa et al., 2010).

The incidence of footpad dermatitis is increasingly used as an indicator of broiler flock welfare (Dowsland, 2008). Footpad dermatitis evaluation is included in a recently developed European standard protocol for monitoring broiler welfare (Welfare Quality®, 2009). In Sweden and Denmark footpad dermatitis is already routinely assessed manually by trained experts. If the overall flock score, based on the prevalence and severity of the lesions, exceeds certain limits producers have to reduce their stocking density or correct management deficiencies (Ekstrand et al, 1998; DCAW, 2012).

These compulsory footpad dermatitis monitoring schemes have led to a reduction in prevalence and severity of footpad dermatitis in both countries (Nielsen, 2010). However, the current assessment by trained experts is labor intensive and thus costly (Ekstrand et al., 1998). Furthermore, these experts need to be trained and tested continuously in order to verify and maximize consistency between and within observers (Algers et al., 2001).

In order to reduce labor costs and human errors a prototype image-analysis system for the automatic assessment of footpad lesions at the slaughterhouse was developed by Meyn Food Processing Technology B.V. (Oostzaan, The Netherlands). This automatic system is designed to assess each bird of a flock without slowing down the slaughter process, whereas the manual monitoring in Sweden and Denmark is based on a sample of only 100 birds per flock. A previous evaluation of the automatic system (De Jong et al., 2010), showed that only 4.2% of all birds passed the system without an assessment (due to e.g. wrong positioning in the shackle, or the presence of feathers on the footpads). Thus, replacing human experts by an automatic system could greatly increase the number of birds that can be assessed without an associated increase in labor costs. However, if such a system is implemented it should not only be quick, but it should also assess the incidence and severity of dermatitis reliably, i.e., the automatic scores should correspond with the currently used human expert scores.

The aims of this study were therefore (a) to evaluate the technical working of the automatic assessment system, (b) to test the correspondence between the automatic scores and human expert scores given on-farm and at slaughter, and (c) to use these data to calculate and compare the Swedish/Danish and the Welfare Quality® overall flock scores. Furthermore, we explored some fruitful avenues for further refinement of the automated system by identifying some approaches to reduce the discrepancy between the automated and human expert assessment.

3. Materials and methods

An expert with substantial training and experience selected the experimental birds from a 38-days-old flock of Ross 308 and Cobb 500 broiler chickens (1 to 1 ratio) kept at a research farm under semi-commercial circumstances. Twenty animals (aimed) were selected for each of five classes of footpad dermatitis (**FARM)** as defined by Welfare Quality® (2009). This scale increases from category 0 (no evidence of footpad dermatitis) to category 4 (severe evidence of footpad dermatitis). Within an individual, the footpad category was based on the foot with the most severe dermatitis.

Two days later, these 5 batches of 20 broilers were transported to a commercial slaughterhouse (Flandrex, Ommel, The Netherlands), where the feet were assessed again for footpad dermatitis. First, the feet were assessed by the automatic system (**AUTO**). This prototype automated assessment system of Meyn Food Processing Technology B.V. was installed at the point of the slaughter line where the feet were already separated from the body, but not yet de-shackled. A camera photographed the feet as they passed underneath the system. Using these photographs, image processing software first identified the toes, and then determined the location of the footpad based on the location of the toes. Subsequently, the proportion of dark area (representing dermatitis) within the area designated as the footpad was determined and used for categorizing the severity of footpad dermatitis. These categories were chosen to resemble those of the Swedish 3-point scale for dermatitis with category 0 (no or hardly any evidence of dermatitis), category 1 (mild to moderate lesions) and category 2 (severe footpad lesions). It ought to be noted though that the Swedish system, developed by L. Berg, is not only based on area but also on depth (Ekstrand et al., 1998).

After the automatic assessment, the feet were again analyzed manually (**SLAU**) by the same expert that had performed the on-farm assessment. This second manual assessment was performed blindly, i.e., the expert did not know the scores the birds had received previously, and birds were assessed in random order using the aforementioned Swedish 3-point scale.

The experiment was replicated once with another flock of broilers chickens from the same farm. The study was approved by the ILVO Ethical Committee for animal experiments.

3.1 Technical performance of the automatic system

The photographs and accompanying graphical documentation of the footpad area affected by dermatitis produced by the automatic system were checked for obvious errors one by one. The number of feet assessed per bird was noted and it was checked if the system had assessed the correct location(s) (i.e., not the shades in the border of a footpad, feathers or other dirt). When the system assessed the correct location(s) on a footpad, this footpad is considered to have been assessed 'technically correct'. In addition, we counted the number of individuals for which the wrong (i.e. upper) side of the foot or the wrong footpad (i.e. from a neighbouring chicken) on the shackle line were assessed by AUTO.

3.2 Comparison of individual scores

The concordance between the three footpad dermatitis scores (FARM-AUTO-SLAU) was expressed as the Spearman correlation coefficient and the percentage of animals with the same score. For each pair-wise combination of evaluation scheme (FARM vs. AUTO, FARM vs. SLAU, AUTO vs. SLAU), we also derived the percentage of animals for which the score of the first evaluation scheme was below that of the second evaluation scheme. The sign test was used to assess whether this percentage was different from the percentage expected when there would have been equal probability that the score of the first evaluation scheme was below or above the score of the second evaluation scheme.

To calculate the percentage equal scores, the FARM scores based on a five-point scale needed to be converted to a three-point scale used for the SLAU and AUTO scores. By comparing the definitions of both footpad dermatitis assessment systems it was decided to combine FARM categories 0 and 1 to a new category 0 (comparable to SLAU and AUTO category 0), FARM categories 2 and 3 to the new category 1 (comparable to SLAU and AUTO category 1), FARM category 4 to the new category 2 (comparable to SLAU and AUTO category 2).

The correlation, expressed as the Spearman correlation coefficient, between the three footpad dermatitis scores (FARM - AUTO - SLAU) was calculated not only for the entire dataset but also for the following 3 data sub-sets: 1) only those broilers of which one or both feet were assessed 'technically correct' by the automatic system; 2) broilers of which both feet were assessed by the automatic system; 3) broilers of which both feet were assessed 'technically correct' by the automatic system.

In addition, we compared the dermatitis scores given by the experts according to whether or not the footpads could be assessed by the automatic system. Using a logistic regression model with cumulative logits, we tested whether non-assessable feet lead to systematically higher or lower scores than assessable feet.

3.3 Comparison of flock scores

For the 3 sets of footpad dermatitis scores (FARM – AUTO – SLAU) of the individual chickens, flock level scores were calculated and compared (pooling the two replicates). Two methods for calculating flock level scores were used: the Swedish/Danish (Veldkamp et al. 2007; DCAW, 2012) system and the Welfare Quality® method (Welfare Quality®, 2009). Both methods are based on the percentage of animals in each dermatitis category multiplied by a weighing factor (Table 1). Flock scores were calculated for the entire dataset.

Table 1 Flock-level footpad dermatitis scores calculated according to both existing methods: the Swedish/Danish and the Welfare Quality® system. The flock-level Welfare Quality® score ranges from 0 – 100 with higher scores indicating less dermatitis. The Danish and Swedish flock-level scores range from 0 to 200, and in contrast to the Welfare Quality®, higher flock-level scores indicate more dermatitis. The flock scores are calculated for both broiler batches together.

Flock score	***Flock score formula***
Sweden/Denmark	[% category 1]*0.5 + [%category 2]*2
Welfare Quality®	[(0.50686*I)-(0.0072409*(I²))+(0.000081315*(I³))]
	(I = [% category 1]*0.29 + [% category 2]*1)

	Footpad dermatitis evaluation system		
Flock scores	FARM	SLAU	AUTO
Sweden/Denmark	61.5	66.3	67.6
Welfare Quality®	18.1	12.3	12.4

4. Results

4.1 Technical performance of the automatic system

The automatic system was developed to identify and assess both the right and left footpad of each chicken. The broiler would then be allocated the highest category of both footpads. However, the automatic system only recognized and allocated a score to both footpads for 86 (43.7%) of the 197 birds that passed the system. For 81 (41.1%) of the chickens only one footpad was assessed, and for 30 (15.2%) of the chickens, no footpad was recognized by the system and consequently no AUTO score was obtained. When both footpads were recognized and assessed by the automatic system, the left and right footpad were given the same score for 70.5% of the broilers.

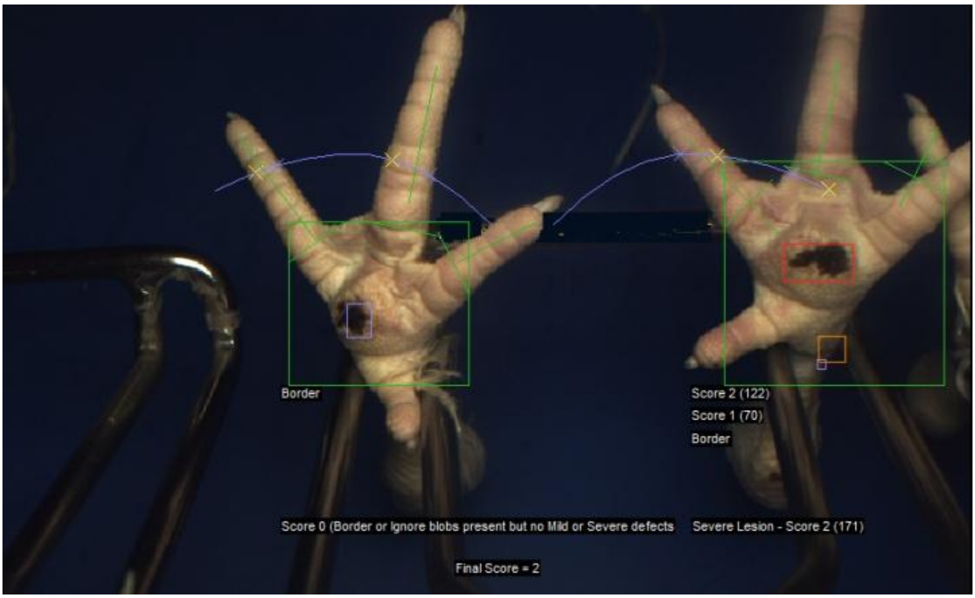

Figure 1 Broiler feet as evaluated by the automatic footpad assessment system. The squares drawn around the dark areas are given a different color depending on the proportion of the area, relative to the area of the green square, and thus indicating the severity of the lesion. Orange squares stand for a dermatitis score 1, red squares are given a score 2 and purple squares indicate dark areas at the border of a footpad, which are not assessed as dermatitis. In this example, the automatic system disregarded the dermatitis on the left foot, whilst assessing a shaded area as dermatitis on the right foot. Consequently, the automatic system allocated a dermatitis score of 0 to the right footpad despite the presence of a considerable lesion. However, as the left footpad was given a dermatitis score of 2, and as a bird always receives the highest category of both feet, the system allocated a score of 2 to this broiler.

Several mistakes concerning the assessment of the dermatitis score of individual footpads were identified. Twice, the upper side of the foot was assessed, and on 3 occasions the wrong (neighboring) footpad was evaluated. Even when the correct side of the correct footpad was considered, several problems were identified. For 49.4% of the broilers, the system indicated dermatitis in places where it was not actually present (e.g., a shaded area instead of the location where the actual footpad lesion was). Figure 1 is taken as an example for explaining frequently occurring mistakes. The automatic system did not consider the area of discoloration identified on the right footpad (purple square) as a footpad lesion but as a border area (because it was on the edge of the footpad). Consequently, the automated system allocated a dermatitis score of 0 to the right footpad despite the presence of a considerable lesion. However, as the left footpad was given a lesion score of 2 and as a bird always receives the highest score of both feet, the automated system allocated a footpad lesions score of 2 to this chicken.

4.2 Comparison of individual scores

The percentage of chickens in each footpad dermatitis category is given in Table 2 and a summary frequency table is given in Table 3. The correlation between the scores given by the trained expert on-farm and at slaughter was stronger than the correlation between either of these expert scores and the automatic score (Table 4). The percentage equal score (i.e., how many times do A and B exactly agree) was better when the two scores given by the human expert were compared, than when the automatic assessment and the expert score given on-farm were compared ($P = 0.0046$). The percentage equal score between the two expert scores did not exceed the percentage equal score between the automatic assessment and the expert score given at slaughter ($P = 0.20$). However, none of the assessment systems produced higher or lower scores than the other ones (Table 4).

Table 2 Contingency table for SLAU and AUTO scores for the whole dataset and for the group of broilers of which both feet were assessed 'technically correct'. The sum of the row percents for a specific footpad dermatitis category given by the expert at the slaughterhouse (SLAU score), equals to 100%.

Whole dataset – both broiler batches together

	SLAU	AUTO 0	AUTO 1	AUTO 2	Missing Data	Row Totals
Count	0	48	9	3	17	77
Row Percent		62,34%	11,69%	3,90%	22,08%	
Count	1	15	37	10	10	72
Row Percent		20,83%	51,39%	13,89%	13,89%	
Count	2	8	9	27	3	47
Row Percent		17,02%	19,15%	57,45%	6,38%	
Count	Missing	1	0	0	0	1
Row Percent		100,00%	0,00%	0,00%	0,00%	
Count	All Grps	72	55	40	30	197

Both feet assessed 'technically correct'

	SLAU	AUTO 0	AUTO 1	AUTO 2	Missing Data	Row Totals
Count	0	14	1	0	0	15
Row Percent		93,33%	6,67%	0,00%	0,00%	
Count	1	1	12	2	0	15
Row Percent		6,67%	80,00%	13,33%	0,00%	
Count	2	0	0	7	0	7
Row Percent		0,00%	0,00%	100,00%	0,00%	
Count	Missing	0	0	0	0	0
Row Percent						
Count	All Grps	15	13	9	0	37

Table 4 Presence of the different footpad dermatitis categories (in percentages) evaluated by an expert at the farm (FARM) and at the slaughterhouse (SLAU) and by the automatic system (AUTO), according to the differently composed groups: the whole data set; one or both feet assessed and all assessed feet or only the assessed feet 'technically correct'.

		Presence of the different footpad dermatitis classes (%)														
		Whole group of evaluated broilers			Broilers with only one footpad assessed by the automatic system			Broilers with both feet assessed by the automatic system			Broilers assessed 'technically correct'			Broiler with both feet assessed 'technically correct'		
FARM category	SLAU-AUTO category	FARM	SLAU	AUTO	FARM	SLAU	AUTO	FARM	SLAU	AUTO	FARM	SLAU	AUTO	FARM	SLAU	AUTO
category 0	category 0	20.4	39.3	40.3	14.8	35.0	49.4	20.9	37.2	37.2	19.3	42.2	47.0	21.6	40.5	40.5
category 1		19.4			19.8			17.4			21.7			18.9		
category 2	category 1	19.9	36.7	34.6	16.0	33.8	25.9	20.9	40.7	39.5	18.1	36.1	30.1	16.2	40.5	35.1
category 3		19.4			19.8			24.4			20.5			24.3		
category 4	category 2	20.9	24.0	25.2	29.6	31.3	24.7	16.3	22.1	23.3	20.5	21.7	22.9	18.9	18.9	24.3

Table 3 Spearman correlation coefficients and percentage equal scores between FARM, SLAU and AUTO and the percentage assessments for which the first evaluation scheme led to a higher score than the second evaluation scheme. The P-value is related to the hypotheses that one evaluation scheme has a significantly higher or lower score than the other evaluation scheme.

Scheme 1 – Scheme 2	**Correlation coefficient**	**% Equal score (95% CI)**	**% Scheme 1 > Scheme 2**	**% Scheme 1 < Scheme 2**	**P-value**
FARM - SLAU	0.83	77.0 (71;83)	9.7	13.3	0.37
AUTO - FARM	0.54	61.4 (54;69)	20.5	18.1	0.18
AUTO - SLAU	0.59	67.5 (60;75)	19. 3	12.2	0.22

The correlations between the automatic score and the expert scores were improved when sub-sets of the full dataset were used including only those broilers for which the automated system made no mistakes in the marking of dermatitis areas and/or for which it succeeded in assessing both feet (Table 5). In fact, when the automatic system assessed the correct area on both feet the correlation coefficient between the automatic and expert scores was even higher than the correlation coefficient between the expert's on-farm and at-slaughter scores. Conversely, correlation coefficients were much reduced when only broilers were considered for which only one of both footpads was assessed by the automatic system ($r_{(AUTO-FARM)} = 0.40$ and $r_{(AUTO-SLAU)} = 0.43$; $P < 0.0001$).

Footpads of individuals for which the automatic system had not produced a dermatitis score were more likely to be given a high dermatitis score by the expert at the farm and at the slaughterhouse (FARM: odds = 2.32, P = 0.03 and SLAU: odds = 2.46, P = 0.02).

Table 5 Spearman correlation coefficients between FARM, SLAU and AUTO for those broilers where both feet were assessed by the automatic system and for broilers where no erroneously areas were marked and assessed on the footpad ('technically correctly' assessed feet) by the automatic system. The last column shows the result for a combination of both assessments.

Concordance			
Scheme1–Scheme2	Broilers assessed 'technically correct' n=83	Both feet assessed by the automatic system. n=86	Both feet assessed 'technically correct' n=37
FARM - SLAU	0.83	0.82	0.85
FARM - AUTO	0.68	0.71	0.82
SLAU - AUTO	0.74	0.77	0.91

4.3 Comparison of flock scores

Table 1 shows the overall flock scores derived from the 3 sets of footpad lesion scores for the individual chickens (FARM – AUTO – SLAU) according to the formulas used by the Swedish/Danish and the Welfare Quality® system. By numerically comparing the three sets of scores, it was found that regardless of the compilation method used, the flock scores based on the automatic assessment were very similar to those based on expert assessment at slaughter. However, when the Welfare Quality® method was used to compile the scores, the flock-level on-farm expert score was higher than the flock-level at slaughter expert score or the automatic score.

5. Discussion and conclusion

This study shows that individual footpad dermatitis scores provided by the automatic prototype system did not agree well with scores given by a human expert, despite them resulting in overall flock scores of similar magnitude. There are several indications that this poor agreement at the level of the individual chickens is mainly attributable to shortcomings of the automatic system.

The scores given by the expert are likely to be highly reliable. Although the correspondence of our expert with other experts was not compared in the present study, our expert received training, prior to the experiment, that required a high inter-observer reliability. Moreover, the percentage of feet given the same score twice by the expert in the present study was high (although the time period between the assessments confounded the results), i.e., individual expert scores given on-farm and at slaughter agreed well. It is conceivable that the correspondence between these scores would have been even higher if the time lapse between the first and second assessment session had been shorter. Indeed, during the two days between the first and second assessment session some chickens may have developed (more severe) footpad dermatitis. This is evidenced by the lower Welfare Quality® overall footpad dermatitis score of both entire flocks for the on-farm versus at slaughter assessment by the expert (Table 1).

The automatic assessment of footpads at slaughter could be a very useful tool. Unfortunately, the agreement between the expert (human) assessment and the automatic system was poor if the entire dataset was used. This discrepancy may be due to the automatic system using only area of discoloration for the allocation of dermatitis score, whereas the Swedish/Danish system used by the human experts is based also on the depth of the injury. However, as the automatic system, when performing technically correct, has a high concordance with the human expert scores, there may be a sufficient correlation between area of discoloration and depth of injury to allow only the former to be used. This would need to be evaluated on a larger sample than the 37 feet included here, and where area of discoloration is measured manually before expert assessment is carried out. This would allow us to disentangle the correlation between true footpad dermatitis score (as assessed by the human experts) and true area of discoloration from the correlation between true and automated measurement of discoloration.

Nevertheless, even if the automatic system cannot use information about the depth of the dermatitis wound a high concordance with the scores given by a human expert can be achieved. Indeed, restricting the dataset to footpad scores that had been determined by the automatic system without obvious technical shortcomings, considerably improved the concordance between the automatic scores and the expert scores. The greatest improvement was realized by considering only those 43.7% of the chickens for which the automatic system produced a dermatitis score for both footpads. For nearly one-third of the chickens, the automatic footpad score differed between the left and right leg. Contrary to Berg (2004) who reported that both footpads usually show the same degree of dermatitis, De Jong et al. (2011) showed that footpad dermatitis scores can differ considerably between the left and right footpad.

A slightly smaller, but still considerable, improvement in the concordance between automatic scores and expert scores was obtained by omitting the 49.3% of the broilers for which the image analysis software had not correctly recognized the area of the footpad affected by dermatitis. Combining both strategies (excluding birds without both footpads assessed and without correctly recognized dermatitis areas) resulted in a very high correlation with the expert scores.

Despite these discrepancies in the footpad dermatitis scores of individual chickens produced by the automatic system versus the expert assessment at slaughter, the overall flock score did not differ greatly irrespective of whether the Swedish/Danish or the Welfare Quality® system was used. This seems to indicate that, at least for the population examined in the present study, the automatic system, does not consistently over- or underestimate the severity of footpad lesions as compared to the expert. Nevertheless, we identified at least one potential source of systematic bias: feet for which the automatic system failed to produce a dermatitis score were more likely to have been given a high score by the expert. At present we can only speculate about the possible cause of this bias. One possibility, which we will investigate in a follow-up study, is that heavier broilers also have thicker legs that may not fit very well in the shackles used in the slaughterhouse. This may affect the angle at which they are presented to the camera of the automatic system. If heavy broilers' feet pass the system undetected more often, this could explain why birds with a worse footpad category pass undetected more often, as body weight and footpad dermatitis have been reported to be positively associated (Harms and Simpson, 1975; Mayne, 2005), although others reported no such association (Buffington et al., 1975; Martland, 1985; Renema et al. 2007; Mendes et al., 2011).

The study has shown that the footpad dermatitis scores given by the human expert to individual chickens agree rather poorly with the scores produced by the prototype automatic system as it is used at present. Nevertheless, the study also showed the potential of such an automatic system that allocates a footpad dermatitis score by estimating the proportion of the footpad that is discolored even if a human observer may use different or additional information for allocating a dermatitis score. Correspondence with expert scores could be improved considerably if the automatic system manages to assess both feet of a higher proportion of the broilers and if fewer mistakes are made in identifying the area of the footpad affected by dermatitis. The apparent bias between the severity of footpad dermatitis and the likelihood of being missed by the automatic system needs to be investigated further.

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CHAPTER 6:

GENERAL DISCUSSION AND CONCLUSION

1. The importance of improved assessment tools for thirst and footpad dermatitis

Currently, broiler meat producers in Belgium are rearing chickens in large closed poultry houses where the environment is fully controlled. They are kept under intensive and artificial conditions (Sørensen et al., 2006; Van Horne et al., 2008; Vlaamse Overheid, 2012). The birds that are reared within these systems have been selected for extremely fast growth (Havenstein et al., 2003; Meluzzi et al., 2009). As a result, poultry meat can be produced cheaper than red meat or pig meat. It is expected that poultry meat in the near future will become the first meat production worldwide (FAO, 2011). This selection for fast growth combined with the intensive housing conditions, are recognized as the main factors that compromise the welfare of broilers (Meluzzi et al., 2009; EFSA, 2010). Also climatic, environmental, nutritional, physiological, physical, social or psychological stressors are known to reduce welfare (Freeman, 1985).

Welfare problems do exist in the broiler production system and although these problems are scientifically established (EFSA, 2010), better assessment tools are crucial to improve animal welfare in the long run. Moreover, besides the problem of animal welfare as such, European consumers increasingly prefer animal-friendly products and claim to be willing to pay premium prices for it (i.e., value-added product) (Moynagh, 2000; European Commission, 2006). Consumers have sometimes difficulties finding out which products are produced animal-friendly. To help the consumers, labels indicating the welfare levels can be created. Vanhonacker et al. (2009) explain that such labels may contribute to lowering search efforts for products with better (compared to standard) animal welfare levels. However, in order to be able to evaluate and compare animal welfare levels in different stages of the broiler production process (i.e., on farm, during transport and at the slaughterhouse), reliable, feasible and valid assessment schemes are needed. Otherwise, false allegations of a welfare added value can be claimed.

Tools for assessing the welfare of broiler chickens should use a wide range of indicators (SCAHAW, 2000). These indicators should be objective, sensitive, easy to assess under audit conditions and should represent an accurate reflection of animal welfare (Sørensen et al., 2001). An assessment approach founded on animal-based indicators, although still work in progress, is very promising. It offers the possibility of assessing animal welfare more directly in terms of their condition, health, performance and behavior (Gloor et al., 1985; Leeb et al., 2001; Whay et al., 2003; European Parliament, 2009). In addition, resource- and management-based indicators related to design (e.g., stocking density) and micro-environment (e.g., ammonia level), even if they are reliable and easier to measure, can only identify conditions which could be detrimental to animal welfare. They do not reflect poor welfare in animals per se (Sevi, 2009; Blokhuis, 2012). An advantage of animal-based indicators instead of resource-based indicators is that it gives a farmer more 'freedom' to design an optimal environment, as the animal indicates if it is sufficient in term of welfare or not (I. de Jong, personal communication, 2013).

Several research initiatives for animal-based welfare assessment started. An example of such a newly developed scheme is the Welfare Quality® protocol for broilers (European Parliament, 2009; Blokhuis, 2012). A problem within the Welfare Quality® assessment system is that some important aspects are still assessed by resource- and/or management-based indicators.

One such example is the assessment of thirst. Freedom of thirst is of paramount importance for animal welfare and within the Welfare Quality® protocol it is given a lot of weight relative to other welfare criteria when integrating the scores for the separate welfare measures into an overall welfare category (Brambell, 1965; Vanhonacker et al., 2008; Tuyttens et al., 2010). In Welfare Quality® and other broiler welfare evaluation schemes, however, thirst is still assessed by a resource-based indicator (e.g. number of animals per drinker) (Sprenger et al., 2009) due to the lack of a good animal-based indicator. Because the sensitivity and validity of such resource-based indicators are questionable, we focused in this dissertation on the development and validation of animal-based indicators of thirst in broiler chickens (Chapter 3 and Chapter 4).

Apart from having reliable, valid and feasible animal-based indicators for assessing the welfare of broiler chickens, one should also consider the achievability of the entire assessment protocol. The application of such protocols should be labor and time efficient. Otherwise they are likely to become very costly which will prevent their adoption by the industry. Stakeholders already expressed their concern on the amount of time needed to perform the animal welfare assessment protocols (Manten et al., 2011). Technologies for automated recordings of animal welfare need to be further developed in order to have less direct human auditing. Human auditing is considered to be more time consuming, less objective and less reliable. There is a need for more systematic flock monitoring and surveillance programmes in the broiler industry. Visual inspection (can easily be integrated into the slaughter process) has a very high potential to improve animal welfare in broiler production when a range of appropriate animal-based measurements are used in the slaughterhouse (EFSA, 2012).

Imaging inspection has a high potential to improve animal welfare assessment in broiler production when a range of appropriate animal-based measures are integrated into systems within the slaughterhouse (De Jong et al., 2008; EFSA, 2012). Potential advantages of 'automated assessment of on-farm animal welfare' compared to 'on-farm auditing' are that they record real-time and assess welfare more objectively. Furthermore, biosecurity risks associated with farm visits can be avoided (European Parliament, 2009). Introducing automatic assessments at the slaughter line, would greatly improve the (time and labor) efficiency, objectivity and would avoid selection bias (random selection of animals to be tested). Next to these advantages, also larger samples could be evaluated because with human assessment, labor and time constraints limit the number of broilers assessed per flock (De Jong et al., 2011).

The field of automated recording of animal-based (welfare) parameters is relatively new. Some electronic tools are currently available to farmers (e.g. automatic weighing of broiler chickens). But, most of these tools and the associated research efforts focus on specific research goals (e.g., developed for laboratory animals) or production-related parameters, rather than welfare parameters (Blokhuis, 2009). Effort has been done on automation of monitoring locomotary problems, e.g. Dawkins et al. (2009) investigated optical flow patterns in broiler chicken flocks as automated measures of gait, or Nääs et al. (2010) investigated the automation of assessing locomotion deficiencies in broiler chickens. Within the Welfare Quality® project a prototype for automatic assessment of footpad lesions in broilers was developed (De Jong et al., 2008). This system was developed in collaboration with the industry and is based on existing imaging techniques used to monitor aspects of carcass classification. As footpad lesions are a major welfare concern in broilers that can be assessed at the slaughterhouse and as time constraints limit the number of birds per flock that can be assessed by a human assessor, the second focus of this dissertation is on the evaluation of this automatic assessment system for footpad dermatitis (Chapter 5).

Below we discuss the main results and future prospects of the research on the development of animal-based thirst indicators (section 2) and on the evaluation of the automated system for assessing footpad dermatitis (section 3). In section 4, the general conclusions and future prospects of this work are discussed.

2. Animal-based measures of thirst

Prior to this dissertation, very little research had been conducted on the development and validation of animal-based indicators of thirst on farm or at the slaughterhouse. For this dissertation, four experiments were conducted to evaluate putative behavioral and physiological indicators of thirst in broilers and to assess the usefulness of a newly developed water consumption test to assess thirst.

Plasma chloride concentration could be used as an indicator of short-term or longer period dehydration in broiler chickens. After 6h water deprivation, a significant increase in chloride was observed. However, the level of chloride did not show considerable changes after longer deprivation, which makes it impossible to determine the degree of deprivation the bird was subjected to. The most important, however, is to assess whether birds are dehydrated, rather than the degree of dehydration.

Whole blood sodium concentration showed the most consistent relation with water deprivation duration. Therefore, this indicator can possibly be used to determine the degree of dehydration the bird was subjected to. This relation of sodium concentrations and thirst is supported by many other studies in layers and broilers (Chamblee et al., 1982; Koike et al. 1983; Arad et al., 1985; Chamblee et al., 1988; Robinzon et al., 1990; Swayne et al., 1991; Knowles et al., 1995). Unfortunately, its sensitivity as an indicator of long-term (48h) water deprivation is limited as only 70% of dehydrated birds would be detected even when 10% false positives are allowed.

Plasma creatinine and hematocrit levels in the blood showed opposite reactions to short (6h) and medium or long water withdrawal (24, 36 or 48h). Due to this opposite reaction, the distinction between thirst sustained on the farm and thirst caused by the catching-to-slaughter interval may possibly be detectable. Apart from the possible ability to distinguish between short- and long-term thirst, plasma creatinine values showed the greatest potential as an indicator of long-term (48h) water deprivation. The indicator showing the highest sensitivity for a long-term (48h) water deprivation period was plasma creatinine. Measuring creatinine levels in broilers would detect over 80% of 48h dehydrated birds if no false positives are allowed, and even all if 20% false positives were allowed. The sensitivity of hematocrit as long-term (48h) water deprivation indicator was low.

Absolute drained blood content seemed suitable for the detection of severe dehydration. Only periods longer than 36h of water deprivation could be detected, but considerable variation between birds was observed, resulting in low sensitivity.

For the evaluation of thirst in a poultry house, spontaneous water intake of broilers was shown to be significantly higher in deprived birds compared to control birds. This effect was in the present study independent from the location of the open drinker in the poultry house and as such strict standardization of this factor is not necessary (possibly neither for the drinker type used in the poultry house and the birds' age and weight). The results indicate that the water consumption test is sufficiently sensitive to discern between control and 12h deprived flocks, in Brazil even for 6h. This supports its validity as a thirst indicator useable in a commercial setting. It is, however, important to notice that these results were based on comparative studies in which deprived and non-deprived birds were compared within farm, whereas in practice the water consumption of a group of birds at that very moment will be assessed. In our experimental studies, all factors except the deprivation factor were kept equal between the deprived and non deprived birds, due to randomization, and there is no need to use a threshold level. In practical circumstances, however, no such control group will be available, and the decision of thirst will be made based on a threshold value of water consumption.

Data from the Brazilian farms indicate that the amount of water consumed by broilers able to drink freely for a long period, was dependent on indoor climatic variables, which was not the case in Belgium. This suggests that these variables may have to be considered when interpreting the test-outcome in terms of the thirst level experienced by the broilers. Management and climatic variables as well, should possibly be considered as risk factors for thirst. If such a test will be performed within a welfare assessment scheme (i.e. without applying water deprivation prior to testing) the interpretation of the amount of water consumed during the test (in terms of the amount of thirst experienced by the birds) may have to be adapted according to the breed and the climate inside the housing unit. Therefore, threshold values above the amount of water consumed during the test that indicate thirst ought to take into account these confounding variables.

The previously described physiological indicators of thirst in broilers could also be used in assessment schemes on farm. However, blood sampling could be carried out more rapidly and efficiently in the slaughterhouse, and physiological blood indicators that require chemical analysis in the laboratory may be too time-consuming, impractical or costly for large-scale monitoring applications.

Finally, an indicator for the detection of long-term restricted water access, would be useful for detecting birds that will only drink occasionally. This can occur when severely lame birds stand up to drink only when very thirsty, or when small birds can only access drinkers at specific times (e.g., when other birds lay down near the drinker and can be used as a stepladder, or when water is spilled). If birds are thirsty due to locomotary problems, then a gait score can give us more information. However, not all lame birds are necessary thirsty birds. Birds could also be thirsty for example because they have difficulties reaching the drinkers due to a very high stocking density or because they have difficulties reaching or operating the drinkers. The aim of this test would be to detect the dehydrated birds and not just the lame birds that became dehydrated due to their locomotary problems. Therefore we are not assessing lameness in the birds, but we are investigating other possible tests that can detect thirsty birds, whatever the cause of thirst may be. Body weight was affected by the long-term restricted water access period, however, it can be explained by the lower feed-uptake that appears when water supply is limited (Feddes et al., 2002). Unfortunately, body weight can hardly be used as long-term restricted water access indicator because it is also related to different other welfare problems, e.g. health problems. Regrettably, none of the other investigated indicators (skin turgor, drained blood volume and capillary refill time) was significantly affected by long-term restricted access (as compared to controls), although absolute drained blood content and capillary refill time decreased numerically after long term deprivation.

Future research opportunities resulting from this study

Feasibility of application under commercial circumstances is of great importance for the adoption of animal welfare monitoring schemes by the industry. Chemical analysis in the laboratory is too time-consuming, impractical or costly for large-scale monitoring applications. However, some of the physiological indicators tested in this dissertation are useful for research applications as they have been shown to be valid measures of water deprivation. Therefore, it would be valuable to develop easy to apply, not too costly methods for measuring these indicators at the slaughter line, e.g. develop a sort of test strip for blood that changes color (through binding with another agent) if the concentration is beyond a certain threshold.

Protocols with more easily assessed indicators, e.g. the absolute drained blood content and the capillary refill time, would make them more feasible. These tests could be performed cost-effectively at the slaughterhouse. However, these indicators should be optimized if they are to be used in practice to evaluate dehydration, as their sensitivity is currently poor. Although absolute drained blood content and capillary refill time decreased numerically after deprivation, none of the two was affected by long-term restricted access. It was proposed that providing access to a familiar drinker twice a day for 10 minutes, was enough for creating a realistic simulation of chronic thirst in broiler chickens. However, maybe this is not the correct method for the creation of real chronic thirst in broilers and therefore these indicators were unable to detect this welfare problem. Further research is needed to develop tests to find chronically deprived birds. One could evaluate whether increasing the water restriction period (compared to our investigated 10d water restricted period), assuming by doing this the birds will truly be chronically deprived, would increase the sensitivity of the investigated detection methods.

The behavioral and physiological indicators of thirst in broilers investigated in this dissertation are as yet insufficiently validated as thirst indicator. Further research is needed to investigate the sensitivity of such indicators to different thirst levels or the influence of commercial circumstances, e.g. the effect of feed composition on the test outcome of physiological indicators. Otherwise feed intake could be used to influence thirst assessments.

Once a thirst indicator is proved to be valid and reliable for thirst detection, thresholds for acceptable thirst-levels need to be defined before implementation into assessment schemes is possible. For this, we need to know which parameters should be seen as risk factors and which parameters affect the thresholds. If the water consumption test is to be used to compare thirst levels in flocks of various lines and kept under very different climatic conditions, we recommend further work to clarify the relation between management and climatic variables and the required water intake for homeostasis.

For the water consumption test, which seems to form the basis for an on-farm test included in broiler welfare assessment schemes, the test design should be further optimized. Standardization is needed and we need to determine the optimal number of birds separated and the number of experimental pens in a poultry house needed, as to obtain a representative result for the thirst level present in the poultry house.

In animal welfare assessment schemes, it is important to maximize the sensitivity of a detection test and to minimize the number of ‘false positives’ (here non-deprived chickens that are classified as water deprived) because erroneous penalization may make farmers more reluctant to resolve the problem. More research is required to obtain different threshold values for different sets of management and climatic variables, under which a thirst indicator is assessed.

Possible valid animal-based thirst indicators resulting from our research

The findings on thirst illustrate the potential of animal-based measures to assess different stages of dehydration in broilers. Of all tested physiological indicators, plasma sodium concentration was the most promising thirst indicator as it increased steadily with deprivation duration. Plasma chloride concentration may be more suitable to detect the effects of short-term dehydration and the best indicators of medium-term water deprivation were creatinine and sodium. Spontaneous water intake of broilers shows a lot of promise for an on-farm test of thirst detection. However, further research is needed to validate these results before integration into broiler welfare assessment protocols is possible.

3. Automatically scoring footpad dermatitis in broilers at the slaughter line

For evaluating footpad dermatitis, good scoring systems already exist for a trained assessor (Ekstrand et al., 1998; Berg, 2004; Welfare Quality®, 2009). In this study, the correspondence between the automatic scores and human expert scores given on farm and at the slaughterhouse were compared. For the population examined in the present study, the automatic system does not consistently over- or underestimate the severity of footpad lesions as compared to the expert. The individual footpad dermatitis scores provided by the automatic prototype system, however, did not agree well with scores given by a human expert. There are several indications that this poor agreement at the level of the individual chickens is mainly attributable to shortcomings of the automatic system.

If the entire dataset was used, the agreement between the expert (human) assessment and the automatic system was poor. Two main aspects were identified for improving the correspondence between the expert scores and the automatic scores: 1) identification and assessment of both footpads per individual broiler and 2) correctly recognizing the dermatitis area. When data from incomplete (birds without both footpads assessed) and obviously incorrect (birds without correctly recognized dermatitis areas) assessments were discarded from the dataset, stronger agreement between automatic and expert scores was found.

The discrepancy between the automatic scores and the human expert scores may be due to the automatic system using only the area of discoloration for the allocation of dermatitis score, whereas the expert scores are based on real life assessments in which also the depth of the injury is noticed. Including the depth of a lesion is something an automatic system based on solely 2D video imaging could probably not do. For identifying the depths, a transection of the footpad would be needed, making the development of such hardware more difficult. Nevertheless, even if the automatic system cannot use information on the depth of the dermatitis wound a high concordance with the scores given by a human expert can be achieved.

As for using this automatic prototype system for generating overall flock scores, the automatic system did not differ greatly irrespective of whether the Swedish/Danish or the Welfare Quality® system was used.

Future research opportunities resulting from this study

Automation of the assessment of footpads at slaughter could be very useful. This study showed the potential of such an automatic system that allocates a footpad dermatitis score by estimating the proportion of the footpad that is discolored, even if a human observer may use different or additional information for allocating a dermatitis score. However, the prototype system for automatic dermatitis assessment needs to be improved on several points before it can be considered to replace expert assessment of footpad dermatitis.

As the automatic system, when performing technically correct, has a high concordance with the human expert scores, there may be a sufficient correlation between area of discoloration and depth of injury to allow only the automatic system, based on 2D images, to be used. Once this correspondence between an automatic and expert assessment is high, one could start evaluating the reliability between different experts compared to the automatic assessment. This evaluation has been done by the Animal Sciences Group in the Netherland.

The association between the severity of footpad dermatitis (expert score) and the likelihood of being missed by the automatic system revealed a source of possible bias which ought to be further investigated, if it is still present in the improved automatic system. For example, it should be evaluated whether the missed birds are also heavier and because of their higher body weight hung differently in the slaughter hooks. If the feet are not correctly positioned in front of the automatic system's camera, the camera is not able to make a clear picture of the footpad and consequently not assessed. Another possibility is that those birds with higher footpad dermatitis scores given by the expert, have thicker feet and therefore don't fit nicely into the slaughter hooks, again leading to a missing assessment.

Conclusion on the evaluated automatic footpad dermatitis assessment system

It can be concluded that with the improvements suggested in this dissertation, the automatic imaging detection shows great potential for inclusion in broiler welfare assessment schemes. The first prototype for automatic assessment of footpads at slaughter can be a very useful tool, but correspondence with expert scores should be improved considerably. This could be done by increasing the percentage of broilers of which both feet are assessed by the system and by making fewer mistakes in identifying the area of the footpad affected by dermatitis.

4. General conclusion and future prospects

The society is increasingly sensitive for animal welfare and asks for animal-friendly production systems (Verbeke, 2009). The best way to market value added products is through the use of labels (Vanhonacker et al., 2009) and, therefore, valid and reliable animal welfare assessment schemes are needed. Moreover, these assessment schemes are not only important for labels, they are also needed to improve welfare levels within the whole production system. Billions of broilers are produced all over the world in different types of production systems. We need to be able to control and to compare different production systems using valid animal-based assessment schemes and consequently gain information on animal welfare status on farms, at slaughterhouses or during transport. These results will enable real progress in the field of farm animal welfare in commercial circumstances. Minimum welfare standards need to be guaranteed within broiler production systems. Although several assessment schemes already exist for this purpose, the need for the development of less labor and time intensive schemes is high.

One possibility to improve assessment efficiency is to perform the assessments no longer in the poultry house, but instead at the slaughterhouse (i.e., more broilers can be assessed over a specific time period). In our series of studies, by providing the birds with different periods of water deprivation, we were able to find possible animal-based indicators for thirst at different stages in the broiler's life. Moreover, several of these possible indicators can be applied at the slaughterhouse (e.g. sodium concentration) for the detection of thirst, which makes the assessment more efficient compared to an on-farm assessment.

The slaughterhouse, however, is probably not the most suited place for measuring short-term (i.e., shorter than 6h) dehydration in broilers, because it will only reveal thirst developed within the catching-to-slaughter interval (which can even exceed these 6h). Under commercial circumstances, birds are viz. always deprived of water from the moment that they are being caught at the farm, in order to avoid meat contamination problems during the slaughter process (Bilgili, 2002; Warris et al., 2004). This, however, does not mean that such a short-term indicator is not informative for thirst assessment. Because freedom of thirst is of paramount importance for animal welfare (Tuyttens et al., 2010), indicators should be able to detect different levels of thirst in broilers. Another disadvantage (i.e., for the birds) of measuring thirst at the slaughterhouse, however, is that thirst is measured in broilers that are about to be slaughtered. The obtained information will therefore only be useful for the following flock at the earliest, but not for the flock that was being tested.

Some thirst indicators useful at the slaughterhouse can also have another purpose, next to thirst assessment. They can be applied at the slaughterhouse as a sort of meat quality assessment. For avoiding broiler meat contamination problems, broilers need to be deprived of food and water before slaughter. According to Bilgili et al. (2002), the deprivation time needed for feed withdrawal from the gastrointestinal tract is 5 to 6 hours. An indicator of a minimum 6h water deprivation period can be applied as meat quality indicator. This emptying of the intestines is needed to avoid meat contamination (risk for consumers). If an indicator for a water deprivation period of 6 hours can be found, we can detect through this assessment whether the birds' intestines have had enough time to empty and consequently have a lower meat contamination risk. If this deprivation period of 5 to 6 hours is necessary to avoid meat contamination, than this 6h period can be seen as a necessary harm of distress to the birds. However, this also implies that there is no need to deprive the birds for periods longer than 6 hours. Because causing prolonged thirst to animals is considered to compromise animal welfare, a deprivation period longer than 6h can be considered as unnecessary suffering.

Our proposed physiological thirst indicators (except absolute drained blood content) can also be assessed on living animals on the farm. However, performing these assessments on-farm is less efficient (as described above) and may impose extra stress upon the birds.

On-farm tests are the only option for detecting and consequently improving existing thirst problems within a broiler flock. Moreover, it is important to note that caution is warranted when interpreting the blood indicators of thirst. Most probably, these indicators can be influenced by confounding factors such as feed composition, e.g. adding less salt to the feed. Indeed, if no attention is being paid to feed composition during the life of a broiler, incorrect conclusions could be drawn with regard to thirst.

A spontaneous water consumption test seems more appropriate for an on-farm thirst assessment, because it is a simple test which can easily be implemented in an existing assessment scheme on-farm. The drinkers can be placed within the poultry house and during the water consumption test, other measurements can be performed (e.g. gait scoring, clinical assessment). However, some adaptations to the water consumption test design will probably be necessary (depending on the objectives and required sensitivity) to detect the degree of dehydration within a flock. It is not practical to subject the entire flock to a water consumption test, but testing five birds at four locations within the poultry house is probably not enough for measuring thirst levels of the entire broiler flock. It could be considered to work with several open drinkers - scattered over different locations within the housing unit - that contain water with an aversive component. The aversive component should prevent non-thirsty chickens from drinking this water, such that it can be assumed that more water consumed from the open drinkers indicates a greater number of thirsty chickens within the flock. In this way the need to enclose the groups of chickens subjected to the test may be removed rendering the test less labor intensive and potentially exposing a greater proportion of the flock to the test.

Automation of the welfare assessment can decrease labor costs (by reducing e.g., the time needed for an assessor to be trained to examine the birds, to computerize records) and increase the number of observations compared to a human assessor through increased assessment sample sizes and/or longer assessment periods . This is exactly what the automatic footpad dermatitis system does, provided several adjustments are carried out as to improve correspondence with a human expert assessor. Other advantages of automating the welfare assessment are that the evaluation is done more objectively and transparent, and it further facilitates the possibility to integrate the assessment outcome into an automatic feedback system. Slaughterhouses provide a broiler producer with information on causes of why some birds were rejected as meat product. This reject information together with the footpad dermatitis assessment outcome would provide broiler producers with a more complete view on the quality (welfare and health status) of their produced broiler flocks. Broiler producers should benefit by decreasing the prevalence of footpad dermatitis in their houses, since it affects their financial results (Dowsland, 2008; Veldkamp et al., 2007; SCAHAW, 2000). These are aspects that will probably be welcomed by producers and retailers.

An automatic feedback system for the broiler producers can also be used for taking management actions for improving the welfare of the subsequent broiler flocks. Such management actions already resulted in improved prevalence in Northern countries. Management parameters linked with the identified (through the automatic assessment) levels of welfare problems need to be adjusted in the following broiler flock(s) as to improve welfare levels of broilers reared by this producer. For example, decrease stocking density within the poultry house (allowance of 33 or 39 or 42 kg/m² defined by the EU Directive CD 2007/43/EG) if a high prevalence of severe footpad dermatitis (score 2) is present within a broiler flock.

Water consumption is currently already automatically assessed in poultry houses rearing broiler chickens (VEPEK, 2012). Broiler producers use the water to feed ratio (both automatically distributed) to check whether water supply is adequate, because otherwise the growth rate will decline (Ross, 2009; Cobb, 2012). The optimal water to feed ratio is an estimate calculated on water and feed consumption according to environmental parameters and the age of the bird (Watkins et al., 2009). It does not reflect the actual need of a bird at that specific moment. The Welfare Quality® consortium decided to assess Absence of thirst by comparing the actual number of drinkers in a poultry house with a recommended number of drinkers for the amount of birds present in the house, dependent on the used drinker type (nipples, bells or cups) (Welfare Quality®, 2009). They calculate the criterion score for Absence of thirst based on this compliance with recommendation (dependent from drinker type). This calculation, however, does not take external factors into consideration that influence water consumption, i.e. whether water line pressure is appropriate for the age of the birds, drinker line height, nipple design or water quality (Singleton, 2004; Watkins, 2008; Watkins et al., 2009). Due to these external factors, according to producers (personal communication), the number of drinkers that are necessary in a poultry house changes, making the compliance with recommendation erroneous. The results of our last experiment on water consumption as thirst indicator illustrate the more general discrepancy between the outcome of the animal-based water consumption test and the resource based Welfare Quality® score.

For assessing overall broiler welfare, the Welfare Quality® broiler protocol assesses 12 criteria. Absence of prolonged thirst is one of those criteria and is assessed through a resource-based indicator (i.e. birds per drinker). On the other hand, the protocol also evaluates the criterion Absence of disease by assessing dehydration (among other parameters) of the birds at the slaughterhouse. The given reason is that “dehydration is a state in which the tissues of the bird become deficient in water – usually as a result of disease, making the bird unable to access water supplies, but also potentially (more rarely) due to failure of provision of water" (Welfare Quality®, 2009). Because it is an animal-based indicator of thirst already assessed at the slaughter line by veterinary inspectors (Welfare Quality®, 2009), the question raises why this indicator is not used for evaluating Absence of thirst instead of Absence of disease. A possible problem is that the data collected for pathologies and rejects in many countries and slaughterhouses is not uniform. Slaughterhouses collect this information in 'their own way', which makes it difficult to compare information from different slaughterhouses and countries. In Belgium, as in the Netherlands and Brazil, slaughterhouses do not collect information on dehydration and also don't know how to collect this information. In a perfect situation, all slaughterhouses (globally spread) would agree on 1) the type of pathologies they collect and 2) how to collect them.

A cause of rejection that veterinary inspectors often do collect at the slaughter line is the 'abnormal color' of the broiler carcass. This pathology/abnormality can be noted by an assessor as rejection due to dehydration. Abnormal color is often a sign of dehydration, but can also be a sign of poor bled out (personal communication, Andy Butterworth). This raises doubts on using this animal-based indicator as a valid and reliable thirst indicator for broiler chickens. If a valid method for the detection of dehydration at the slaughter line does exist and is already used as a meat rejection cause within a specific country, then this method will also be useful as an indicator for thirst at slaughter.

In this doctoral study we focused on the assessment of thirst and footpad lesions. Apart from these two important welfare aspects, there are other opportunities that are worth investigating for improving the Welfare Quality® broiler welfare assessment protocol. Some of these opportunities are listed below.

An animal-based indicator for ease of movement. Ease of movement is one of the 12 welfare criteria defined by Welfare Quality® and currently evaluated through a resource-based indicator, i.e. stocking density. However, stocking density gives us no information on how the birds perceive the amount of space they have for movement. The stocking density is often pinpointed as a factor affecting broiler welfare. Consequently, if poor welfare is detected in a poultry house, lowering the stocking density is often suggested as a remedial action. An animal-based indicator for evaluating how broilers perceive their space to move around the poultry house is more appropriate. A refilling test (Vanderhasselt et al., 2012) may form the basis of an animal-based indicator. Such a test may be promising, however, no conclusions on space preference can be made as yet.

Replacement of a categorical scale by a continuous scale with anchor points for scoring the severity of welfare indicators. For many indicators assessment is done according to a categorical scale, e.g. footpad dermatitis: 0 = no lesion; 1 = moderate lesion; 2 = severe lesion. Replacing it by a Visual Analogue Scale (VAS) with anchor points, could possibly improve reliability between observers, feasibility and sensitivity (Tuyttens et al., 2009).

Chickens are by nature animals that live in small groups in which a social hierarchy exists which is probably based on individual recognition (SCAHAW, 2000). No valid indicator(s) has been identified, as yet, for evaluating the expression of social behaviors in broiler chickens. There are some indicators defined in Welfare Quality® for layers, i.e. aggressive behavior, plumage damage and comb pecking wounds, however, these behaviors do not often appear in the young broilers. It is not because they do not often happen within a flock that they should not be taken into account. One could suggest that when such previously described behaviors do appear within a flock, they should have a negative impact on the principle score of appropriate behavior (1 out of 4 welfare principle scores).

The thresholds defined by Welfare Quality® qualification system for the different welfare categories should be evaluated and possibly made more accurate. Through this qualification system, farms are awarded a welfare category of excellent, enhanced, acceptable or not classified. Our observations, however, seem to reveal that all our evaluated farms are classified acceptable, even though there were clearly welfare differences between the different evaluated farms. Perhaps a sub-division within the lower welfare categories could differentiate different broiler farms. This differentiation is probably necessary to motivate broiler producers. If they are all classified together, even if obvious welfare differences appear, they might be less willing to improve welfare levels on their farm.

The Welfare Quality® assessment scheme for broilers is a very good beginning, but further improvement through e.g. the use of more animal-based indicators would make this assessment scheme better. Inserting an animal-based indicator for thirst into this system will improve the overall welfare assessment and if an updated automatic footpad dermatitis system is implemented, this welfare assessment scheme will be more time and labor efficient. Finally, the goal is to achieve a much improved welfare assessment system that will be practical and worldwide accepted by all stakeholders within the broiler production sector.

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SUMMARY

Objective and reliable evaluation of broiler welfare levels in different production systems is important to enable comparison and aims to improve the level of welfare within the broiler production sector. It has already been raised and scientifically validated that problems do exist within the broiler production sector and that these problems need to be addressed. In addition, one should not forget that this is an important and efficient industry for the production of animal proteins which will grow significantly in the future.

Chapter 1 of this dissertation begins with creating an overall picture of the broiler sector. It explains which steps are necessary within the production chain as to obtain the end-product: broiler meat. At Belgian level, information is provided on farm sizes, existing laws regarding broiler welfare, technical details on broiler production aspects and some key economic data. The position of Belgium within the international scene is described as well as the importance, globally, of the broiler production sector in terms of trade and income, and the perception of different stakeholders on animal welfare. The advantages and disadvantages of the broiler production are clarified.

Some definitions on animal welfare are described. The existence of many definitions is caused by the multidisciplinary aspect of animal welfare. In this dissertation the focus was on the operational definition of animal welfare proposed by the European project Welfare Quality® in 2009. Once a clear definition exists, one can start with the evaluation of animal welfare. The welfare problems existing within the broiler industry are explained together with possible tools for assessing these welfare problems. The Welfare Quality® assessment method for broilers is clarified, and some attention points on animal welfare assessment tools are explained.

In this dissertation, it was decided to work on two possibilities for improving welfare assessment schemes. A first improvement can be obtained through the integration of an animal-based indicator for thirst assessment. Secondly, welfare assessment schemes can be improved by assessing existing animal-based indicators automatically, e.g. automatic assessment of footpad dermatitis on the slaughter line. Prolonged thirst and footpad dermatitis are both seen as major welfare problems within the sector.

The main objective of this dissertation was to improve existing assessment schemes for broiler welfare, e.g. the Welfare Quality® broilers protocol, by offering alternatives.

For this purpose, the following objectives were defined in Chapter 2.

First, to identify a reliable animal-based indicator of thirst that can easily be implemented into the quality control system in the slaughterhouse.

Secondly, to examine a spontaneous water consumption test as animal-based indicator of thirst in living broiler chickens under (semi-)commercial circumstances.

Third, to evaluate a first prototype for an automatic assessment of footpad dermatitis at the slaughter line.

In Chapter 3, research was conducted in order to find a reliable animal-based thirst indicator for broiler chickens, which could be integrated into the slaughter process.

This indicator should provide us with information on thirst levels on farm and during the catching-to-slaughter interval. Through a literature review, several parameters were selected which showed a correlation with thirst/water deprivation: skin turgor, capillary refill time, drained blood volume, plasma osmolality, whole blood sodium concentration, plasma chloride and creatinine concentration and blood hematocrit levels. Their accuracy as possible thirst dehydration was examined through the application of different water deprivation periods: a 0 (control), 6, 12, 24, 36 and 48 hour water deprivation period and a 10 day water restriction period.

Whole blood sodium concentration of broilers showed the most potential as thirst indicator due to its gradual increase with increasing water deprivation period. Other parameters showed potential as thirst indicator for the detection of a specific thirst interval. Plasma creatinine broiler blood levels is a potential indicator for differentiating broilers deprived for a short period (6h) from broilers that experienced a long-term water dehydration period (minimum 24h). Plasma chloride concentration showed potential for the detection of a dehydration period from 6h onwards.

An indicator for the detection of a long-term water restricted period in broiler chickens was not identified. Although decreased levels of absolute drained blood volume and capillary refill times were observed after a 10 day water restricted period in broilers, these differences were not found significant.

The sensitivity of the examined physiological blood parameters as detection method if used in practice, was evaluated through Receiver Operating Curves (ROC). Such graphs depict the percentage of false positives when a specific detection method is used. This percentage of false positives generated through a detection method should be absent or limited in order to avoid false penalizations. ROC were created for the detection of a 6h and a 48h water deprivation period. Plasma chloride seemed to be the best indicator of a 6h water dehydration period. Plasma creatinine concentrations showed a good sensitivity for the detection of a 48h dehydration period in broiler chickens.

In Chapter 4, it was examined whether a simple spontaneous water consumption test could be validated as an animal-based thirst indicator on farm.

This animal-based thirst indicator was already examined in 2009 under experimental circumstances. The water consumption test was found to increase gradually with increasing water deprivation period (maximum investigated deprivation period of 24h).

The objectives were to evaluate a water consumption test under (semi-)commercial circumstances, to find out which factors influenced the test outcome under commercial circumstances, to investigate the sensitivity of this test and to evaluate the correspondence between the Welfare Quality® resource-based thirst indicator and this animal-based thirst indicator.

The location of the drinker within the poultry house was examined and the results showed no influence of the drinker's location on broilers' water intake. Birds could have had preferences for different locations within a poultry house depending on whether or not they were dehydrated. The drinker type used in the poultry house and the birds' age and average body weight was not found to influence the post-treatment water consumption test, and as such strict standardization of this factor does not seem necessary.

The results indicate that the water consumption test is sufficiently sensitive to discern between control and 12h deprived flocks, in Brazil even for 6h, supporting its validity as a thirst indicator useable in a commercial setting. However, data from the Brazilian farms indicate that the amount of water consumed by broilers able to drink freely for a long period, was dependent on indoor climatic variables. This suggests that these variables may have to be considered when interpreting the test-outcome in terms of the thirst level experienced by the broilers.

Management and climatic variables as well, should probably be seen as risk factors for thirst. If such a test will be performed within a welfare assessment scheme (i.e. without applying water deprivation prior to testing) the interpretation of the amount of water consumed during the test (in terms of the amount of thirst experienced by the birds) may have to be adapted according to the genetic line and the climate inside the housing unit. The threshold value for the amount of water consumed during the test ought to take into account such confounding climatic variables.

The results illustrate the more general discrepancy between the outcome of the animal-based water consumption test and the resource based Welfare Quality® score. This underlines the importance of the use of an animal-based indicator for the assessment of a welfare parameter.

Automation of indicators that are included within a welfare assessment system would increase time and labor efficiency of the system. An indicator often used for broiler welfare assessment is footpad dermatitis. Within several broiler welfare monitoring schemes, trained veterinary inspectors assess broilers' footpads for dermatitis at the slaughter line (i.e., in Sweden, Denmark and the Netherlands).

A first prototype of an automatic footpad dermatitis assessment system integrated in the slaughter process was evaluated in Chapter 5. The correspondence between the automatic assessment of a group of broilers and the assessment done by a human expert on farm and at the slaughterhouse was evaluated.

The automatic assessment was weakly correlated with both expert assessments, which were highly correlated.

After a manual assessment of the images taken by the automatic system, several mistakes were identified. Only for 43.7% of all assessed broilers, both feet were identified and assessed (the highest score between both broiler's feet is used as footpad dermatitis score). It is possible that both feet of a broiler chicken have a different degree of footpad lesion and therefore it is important that both feet are always scored, as to avoid erroneous dermatitis scores. Moreover, the assessment was often (49.4%) performed on a wrong detection of the dermatitis area on the foot. The correspondence between the automatic assessment and both expert assessments improved much when only birds of which both feet assessed technically correct, were taken into account.

Based on the results from the correspondence between the automatic and expert assessment, a bias possibly seems to appear within the automatic system. Broilers receiving the highest footpad dermatitis from both expert assessments had a higher chance of being missed (no feet were identified) by the automatic system and consequently these birds were discarded from the flock's footpad dermatitis score.

Despite the bad correspondence between the automatic and expert assessments, no clear differences were identified on the calculated mean flock scores. Flock scores were calculated according to the Swedish, Danish and Welfare Quality® system.

The automatic footpad dermatitis system incorporated at the slaughter line, provided several changes are carried out, shows potential for replacement of a human assessor in the slaughterhouse.

In the concluding Chapter 6 of this dissertation, the results of the different studies are described. The feasibility of including the investigated animal-based thirst indicators in assessment schemes is discussed, as well as the potential of automating different welfare indicators.

To conclude, the welfare Quality® broiler protocol is a good beginning as welfare assessment scheme. However, several elements for improvement can be identified. Including an animal-based indicator for thirst and automating an important animal-based welfare indicator (i.e. footpad dermatitis) would definitely improve the broiler welfare assessment scheme. The ultimate goal is to create a practical and worldwide accepted (by different stakeholders within the production sector) broiler welfare assessment scheme.

SAMENVATTING

Het objectief en betrouwbaar evalueren van het niveau van dierenwelzijn in de vleeskuiken productie is belangrijk om de bedrijven onderling te kunnen vergelijken en heeft ook als doel het welzijnsniveau in de vleeskuiken sector te verbeteren. De vleeskuiken productie sector is een belangrijke en efficiënte sector voor de productie van dierlijke eiwitten die in de toekomst nog sterk zal groeien. Het bestaan van problemen in deze sector werd reeds veelvuldig aangekaart en wetenschappelijk onderbouwd en een aanpak dringt zich op.

Hoofdstuk 1 van dit doctoraatswerk begint met het creëren van een algemeen beeld van de sector. De verschillende stappen die noodzakelijk zijn in de productieketen om tot het eindproduct *kippenvlees* te komen, worden uitgelegd. Op Belgisch niveau wordt dieper ingegaan op de bedrijfsgrootte, de bestaande wetgevingen betreffende welzijn die belangrijk zijn voor de sector, de technische informatie van de vleeskuiken productie en enkele belangrijke economische cijfers. De positie van de Belgische vleeskuiken productie sector binnen het internationale kader wordt beschreven, alsook het belang van deze sector, globaal bekeken, op het gebied van handel en inkomen en de perceptie van de verschillende belanghebbenden over dierenwelzijn. De voor- en nadelen van de vleeskuiken productie worden verduidelijkt.

Verschillende definities van de term *dierenwelzijn* worden gegeven. Het bestaan van vele definities vloeit voort uit het multidisciplinaire aspect van dierenwelzijn. In dit doctoraat ligt de focus op de operationele definitie van dierenwelzijn die voorgesteld werd in 2009 door het Europese project Welfare Quality®. Zodra er overeenstemming is inzake de terminologie kan men beginnen met het evalueren van dierenwelzijn. De welzijnsproblemen die bestaan in de vleeskuiken sector worden opgelijst en verduidelijkt alsook methoden om deze problemen te beoordelen. De Welfare Quality® evaluatie methode voor vleeskuikens wordt beschreven en de mogelijkheden ter verbetering van dierenwelzijn evaluatie methoden.

Er werd besloten om binnen dit doctoraat, enerzijds te werken aan het vinden van goede diergebonden indicatoren van dorst (ter vervanging van bestaande huisvestingsgebonden indicatoren) en anderzijds aan het evalueren van een in 2008 ontwikkeld eerste prototype van een automatisch systeem dat voetzool ontstekingen aan de slachtlijn kan beoordelen. Zowel de aanwezigheid van dorst als van voetzool ontstekingen binnen een stal met vleeskippen worden beschouwd als belangrijke welzijnsproblemen binnen de sector.

De hoofddoelstelling van dit doctoraat, is de bestaande welzijnsevaluatie methoden voor vleeskuikens te verbeteren (bv. de Welfare Quality® vleeskuikens evaluatie methode) door alternatieven aan te bieden.

De volgende subdoelstellingen werden gedefinieerd in hoofdstuk 2.

Ten eerste, het identificeren van een betrouwbare diergebonden indicator voor de detectie van dorst bij de vleeskippen die gemakkelijk te integreren is in het kwaliteitscontrole systeem dat wordt uitgevoerd op het slachthuis.

Ten tweede, het onderzoeken van een spontane water consumptie test als een indicator voor dorst bij levende vleeskippen onder (semi)commerciële omstandigheden.

Ten derde, het evalueren van een eerste prototype voor een automatische beoordeling van voetzool ontstekingen aan de slachtlijn.

In hoofdstuk 3 werd op zoek gegaan naar een betrouwbare diergebonden dorst indicator voor vleeskippen die geïntegreerd kan worden in het slachtproces (eerste subdoelstelling).

Deze indicator diende informatie te verschaffen over dorst zowel op het bedrijf als tijdens het vangen-tot-slacht interval. Via een literatuurstudie werden enkele parameters geïdentificeerd die gecorreleerd waren met dorst of water deprivatie, nl. huidturgor, capillaire vullingstijd, gedraineerd bloedvolume, plasma osmolaliteit, natrium concentratie in het bloed, plasma chloride en creatinine concentraties en hematocriet bloedwaardes. De nauwkeurigheid van deze parameters als mogelijke dorst indicatoren werd onderzocht onder verschillende water deprivatie periodes: 0 (controle), 6, 12, 24, 36 en 48 uur water deprivatie of een 10 dagen durende water restrictie periode.

De natrium concentratie in het bloed van de vleeskippen vertoont het meest potentieel als dorst indicator vermits deze parameter gradueel stijgt bij een toenemende water deprivatie periode. Andere onderzochte parameters toonden ook potentieel als dorst indicator, maar uitsluitend voor de identificatie voor een bepaald dorstniveau. Zo kan men via het analyseren van de plasma creatinine niveaus in kippenbloed naar alle waarschijnlijkheid een onderscheid maken tussen dorst die kort geleden (6u) ontstond, of minstens 24 uur geleden begon. De plasma chloride concentratie in kippenbloed zou een goede indicator kunnen zijn voor het detecteren van dorst die minstens 6 uur geleden begon.

Een goede diergebonden indicator voor een lange water restrictieperiode werd niet gevonden in deze studie vermits het verlaagd niveau van gedraineerd bloed volume en capillaire vullingstijd waargenomen bij een 10 dagen durende water restrictie periode, niet significant werd bevonden.

Om na te gaan wat de gevoeligheid van de hiervoor besproken fysiologische bloed indicatoren zou zijn als detectie methode voor dorst, werden enkele Receiver Operating Curves (ROC) opgesteld. De ROC grafieken werden opgesteld voor de detectie van een 6 en 48 uur water deprivatie periode. Dergelijke grafieken geven het percentage aan van vals positieve uitkomsten die gegenereerd zouden kunnen worden via een bepaalde detectie methode. Het percentage vals positieven bij een detectie methode moet zo laag mogelijk zijn om onterechte sanctionering tegen te gaan. Voor de detectie van een 6 en 48 uur dehydratatie periode bij vleeskippen, bleken het analyseren van respectievelijk de plasma chloride concentratie en de plasma creatinine waardes in kippenbloed, de beste methoden.

In hoofdstuk 4 werd onderzocht of een spontane water consumptie test bij vleeskippen kon gevalideerd worden als diergebonden dorst indicator op het bedrijf (tweede subdoelstelling).

Deze dorst indicator werd in 2009 reeds onderzocht onder experimentele omstandigheden. Er werd aangetoond dat de waterconsumptie van vleeskippen gradueel steeg met een toenemende deprivatie periode (maximale onderzochte water deprivatie periode was 24u).

In dit onderzoek werd de water consumptie test geëvalueerd onder (semi)commerciële omstandigheden, werden de factoren bekeken die deze test uitkomst beïnvloeden en werd nagegaan wat de gevoeligheid van deze water consumptie test als dorst indicator is. Er werd ook onderzocht of de locatie van de drinker (tegen de muur of in het centrum) een invloed zou kunnen hebben op de waterconsumptie van de dieren en de overeenkomst tussen de diergebonden water consumptie test en de huisvestingsgebonden gebonden Welfare Quality® score voor dorst wordt nagegaan. De hypothese was dat de meer gedepriveerde dieren een voorkeur zouden kunnen hebben voor een bepaalde locatie binnen de stal.

Deze test bleek gevoelig genoeg om het onderscheid te maken tussen controle groepen en groepen die 12 uur gedehydrateerd waren, en in Brazilië zelfs 6 uur. De oorzaak van een verminderde gevoeligheid voor de detectie van 6 uur gedehydrateerde vleeskippen in België kan liggen aan de kortere deprivatie periode, maar ook aan de wijziging in de test opzet, meer bepaald door het meten onder commerciële omstandigheden van de spontane water consumptie over een periode van anderhalf uur i.p.v. twee uur (experiment onder semicommerciële omstandigheden).

De locatie van de drinker in de vleeskuiken stal bleek geen invloed te hebben op de test uitkomst wat suggereert dat deze parameter niet hoeft gestandaardiseerd te worden. Eenzelfde geldt voor het type drinker dat in de stal gebruikt wordt, en voor de leeftijd en het gewicht van de kippen in de stal.

Management- alsook klimaatfactoren moeten waarschijnlijk gezien worden als risicofactoren voor dorst. Indien dit soort test geïntegreerd zou worden in een evaluatie systeem (mn. waarbij de kippen niet gedehydrateerd worden voor de test) zou de interpretatie van de wateropname resultaten (in termen van het dorst niveau dat de vleeskippen ervaren) best aangepast worden volgens de genetica van de vleeskippen en de klimaatsomstandigheden binnen de pluimvee stal.

Uit onze resultaten halen we dat de overeenkomst tussen de diergebonden water consumptie test en de huisvestingsgebonden Welfare Quality® score voor dorst eerder zwak bleek te zijn. Dit onderstreept het belang van het gebruik van een diergebonden indicator voor het beoordelen van een welzijnsparameter.

Automatisatie van de indicatoren die geïntegreerd zijn in een welzijns evaluatie methode, zou de (tijds- en arbeids-) efficiëntie van het systeem verhogen.

Een veel gebruikte en gevalideerde diergebonden welzijnsindicator in vleeskuiken welzijns evaluatie methoden is een score voor voetzoolontstekingen/dermatitis. In enkele vleeskuiken welzijns monitoringssystemen worden de voetzolen gescoord door opgeleide dierenartsen aan de slachtlijn (bv. in Zweden, Denemarken en Nederland).

In hoofdstuk 5 hebben we een eerste prototype van een automatisch beoordelingssysteem van voetzoolontstekingen aan de slachtlijn geëvalueerd. De overeenstemming tussen de automatische beoordeling, een expert beoordeling op het bedrijf en een expert beoordeling aan de slachtlijn werd nagegaan (derde subdoelstelling).

De automatische score bleek slechts zwak gecorreleerd te zijn met beide expert scores, maar de beide expert scores bleken wel sterk gecorreleerd met elkaar.

Na een manuele evaluatie van alle beelden die door het automatisch systeem waren genomen, werden enkele fouten opgespoord in het systeem. Slechts bij 43,7% van alle beoordeelde kippen, werden beide voetzolen gescoord (het is de hoogste score van beide voetzolen die genoteerd wordt). Beide voetzolen kunnen echter een verschillende gradatie van deze laesie vertonen, wat het belang van een beoordeling op basis van beide voetzolen onderbouwt. Daarenboven was de beoordeling van het automatische systeem vaak (49,4%)

gebaseerd op een foutieve markering van de ontstekingsregio op de voetzool van de vleeskip. De overeenstemming tussen de automatische score en beide expert scores verbeterde sterk wanneer gebruik werd gemaakt van een dataset waarin enkel de vogels zaten waarvan beide voetzolen duidelijk correct beoordeeld waren.

Uit de resultaten van de beoordeling van het automatisch systeem, bleek eveneens dat er mogelijk een soort bias in het automatisch systeem aanwezig was. Vleeskippen die van beide expert beoordelingen de ergste laesie toegekend kregen, vertoonden een hoge kans om door het automatisch systeem gemist te worden (dus niet beoordeeld te worden).

Ondanks deze slechte overeenkomst tussen de automatische score en de expert scores, werden er geen duidelijke verschillen gevonden op de gemiddelde voetzool ontsteking groepsscores. Deze groepsscore werd berekend volgens drie bestaande welzijn monitoringssystemen: Welfare Quality®, het Zweedes en Deense systeem.

Uit het voorgaande kan worden geconcludeerd dat dit eerste prototype van een automatisch voetzool-beoordelingssysteem, op voorwaarde dat de nodige aanpassingen (beide voetzolen detecteren en correct evalueren) worden doorgevoerd, potentieel vertoont ter vervanging van een menselijke beoordeling aan de slachtlijn.

In een zesde en laatste hoofdstuk van dit doctoraat, worden de resultaten van de verschillende studies besproken.

De haalbaarheid van het gebruik van de onderzochte dorst indicatoren in de praktijk wordt bediscussieerd, alsook het potentieel van een automatische evaluatie van verschillende welzijnsindicatoren.

We kunnen besluiten dat de Welfare Quality® vleeskuiken welzijns beoordelingsmethode alvast veel potentieel vertoont. Het gebruik van meer diergebonden indicatoren (zoals voor afwezigheid van langdurige dorst) kan deze methode opwaarderen.

Om dergelijk soort welzijn monitoringssyteem haalbaar te maken, wordt aanbevolen om meer te werken met een automatisatie van de verschillende indicatoren, zoals voor de evaluatie van de voetzolen aan de slachtlijn.

Het ultieme doel is om een haalbare, praktisch uitvoerbare en gevalideerde methode te ontwikkelen die het welzijn van vleeskippen op een waarheidsgetrouwe manier beoordeelt.

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